CENTRAL BIOGENIC AMINE RECEPTOR ADAPTATION DURING ANTIDEPRESSANT TREATMENT: STUDIES DIRECTED AT THERAPEUTIC MECHANISMS

By

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This dissertation is dedicated to my mother, father and brothers.

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PREFACE

This dissertation is opened by a general introductory chapter and closed by a chapter that integrates and discusses the relevency of this research to understanding the neurochemistry underlying the pathogenesis and treatment of endogenous depression.

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The therapeutic response to antidepressants is thought to be mediated by progressive changes in central biogenic amine receptor density and sensitivity. The major current hypothesis of endogenous depression has focused on changes in central beta-adrenergic receptors responsiveness. The down-regulation of central beta-receptors by antidepressants during chronic administration appears to be due to enhanced stimulation by increased synaptic levels of norepinephrine. We have found that coadministration of alpha$_2$-receptor antagonists with antidepressants accelerated and enhanced central beta-receptor down-regulation. Thus, the delay in beta-receptor down-regulation during chronic antidepressant therapy appears to be due to a progressive decrease in alpha$_2$-adrenergic receptor inhibition of norepinephrine release and adrenergic cell firing.

The mechanism of the antidepressant-induced down-regulation of $5HT_2$ receptors is currently unknown. We have found that the $5HT_2$ receptor down-regulation is not secondary to changes in availability of serotonin and is independent of the beta-receptor down-regulation since it is not prevented by coadministration of the $5HT_2$ receptor antagonist nefazadone or the beta-receptor antagonist propranolol. The importance of serotonin in depression may be related to its role in modulation of central adrenergic synaptic transmission.

Adrenergic transmission is mediated not only by beta-adrenergic receptors, but also by alpha-adrenergic receptors. $Alpha_1$-receptors are present in significant densities in several different brain regions and often elicit opposite electrophysiologic responses from beta-adrenergic receptors. Thus, net adrenergic transmission in several brain regions may depend on the balance between $alpha_1$ and beta-receptor sensitivity.

We provide evidence that the balance between central beta and $alpha_1$-adrenergic receptor responsiveness is altered after chronic treatment with the prototype antidepressant desipramine. In addition, reserpine, an agent which can induce depression in certain patients, produces an opposite shift in the balance of central beta and $alpha_1$-receptors than does desipramine. We propose a novel hypothesis of depression where the balance between beta and $alpha_1$-adrenergic receptor responsiveness may be central to the etiology and treatment of endogenous depression rather than changes in beta-receptor responsiveness alone.

CHAPTER ONE
INTRODUCTION

Endogenous depression constitutes one of the largest and most debilitating diseases in psychiatry. It has been estimated that endogenous depression affects up to 5% of men and 8% of women in the general population (Winokur, 1978). With such a high prevalence, it is tempting to dismiss depression as simply a normal variation or an extreme reaction to environmental stimuli. However, endogenous depression differs from normal reactive depression in its severity, duration, and lack of association with external provocation. It is usually a chronic disease with recurrent depressive episodes of 2 weeks to 6 months duration. Each episode is characterized by a dysphoric mood associated with several other signs and symptoms such as psychomotor retardation, diminished self-esteem, loss of appetite, decreased energy, loss of libido, decreased memory and ability to concentrate, disruption of sleep, and loss of interest in activities that were previously enjoyable. In addition, depressed patients often have recurrent thoughts of death and suicide, and suicide is common among these patients. Alterations in pituitary hormone release are also found in many cases of endogenous depression. This is most commonly manifested by increased plasma cortisol levels with loss of the normal diurnal variation and suppressibility of cortisol release (Gregory and Smeltzer, 1983).

Therapies which are effective in treating endogenous depression include the tricyclic and tetracyclic antidepressants, atypical antidepressants such as iprindole and mianserin, monoamine oxidase inhibitors, and electroconvulsive shock therapy (ECT). A major problem with all of these therapies is that they require chronic administration for two to three weeks before the onset of their clinical actions, and they are not effective in all patients (Klein and Davis, 1969; Oswald et al., 1972). This delayed onset of action is a major therapeutic problem since there is a significant risk of suicide by depressed patients during the treatment period, frequently necessitating hospitalization for several weeks. Thus, an antidepressant therapy with a more rapid onset of action would be therapeutically beneficial.

Noradrenergic and serotonergic nervous systems in the brain have been implicated in the etiology and treatment of depression. Both norepinephrine and serotonin play important regulatory roles in many parameters affected in this disease. Mood appears to be influenced by noradrenergic and serotonergic transmission (Schildkraut, 1978; Murphy et al., 1978), as do pituitary hormone release (Jones et al., 1976) and sleep patterns (Morgane, 1981). In addition, agents which can precipitate depression in certain patients, e.g. reserpine, deplete central stores of norepinephrine and serotonin (Mendels and Frazer, 1974). The importance of noradrenergic and serotonergic nervous systems in depression is further supported by the findings that antidepressants interact with these systems in brain. The tricyclic and tetracyclic antidepressants have been shown to be potent

inhibitors of presynaptic high affinity norepinephrine or serotonin reuptake (Iverson, 1975; Glowinski and Axelrod, 1964), while the monoamine oxidase inhibitors prevent the degradation of these biogenic amines by monoamine oxidase.

In addition to their interactions with noradrenergic and serotonergic neurons, antidepressants have a number of interactions with various receptor systems in brain and periphery (Table 1). Most of the tricyclic antidepressants, and to a less extent the tetracyclic antidepressants, are potent inhibitors of muscarinic receptors (Fjalland et al., 1977; Snyder and Yamamura, 1977). In addition, antidepressants have variable affinities as inhibitors at α_1 and α_2 adrenergic receptor sites (Sugrue, 1983; Tang and Seeman, 1980), but do not bind to β adrenergic receptors (Scott and Crews, 1983; Crews et al., 1983). Many antidepressants also bind potently to histamine sites (Green and Maayani, 1977; Richelson, 1978), yet generally do not interact directly with dopaminergic or serotonin$_1$ ($5HT_1$) receptor sites (Sugrue, 1983). Several antidepressants are also potent in binding to serotonin$_2$ ($5HT_2$) sites (Crews et al., 1983). Although antidepressants interact directly with a number of different receptor types, no correlation has been found between their binding to these sites and their clinical actions as antidepressants. Moreover, antidepressants interact acutely with these sites, yet chronic administration is required to treat depression.

Because of the delayed onset of clinical action of antidepressants, more recent studies on their mechanism of action have focused on changes in central adrenergic and serotonergic synaptic

TABLE 1: Summary of Interactions of Antidepressants with Central and Peripheral Receptors

Receptor Type	In-Vitro Inhibition (Receptor Binding Studies)	Chronic Administration (Effects on β_{max})	
		Antidepressants	ECT
β	-	↓	↓
α_2	±	↓	↓
$5HT_2$	±*	↓	↑
α_1	±	-	?
$5HT_1$	-	-	-
H_1	+	?	?
ACH	+	-	-
DA	-	-	-

\- = No Effect
± = Weakly Inhibitory
\+ = Inhibitory

* Miaserin Potent
↑ = Up-Regulation
↓ = Down-Regulation

transmission that occur after chronic administration. It has been found that chronic, but not acute, administration of antidepressant drugs or electroconvulsive shock therapy (ECT) down-regulates β-adrenergic and α_2 adrenergic receptors inbrain (Banerjee et al., 1977; Scott and Crews, 1983; Crews and Smith, 1978; Svensson and Usdin, 1978). In addition, chronic treatment with antidepressant drugs also decreases central serotonin$_2$ ($5HT_2$) receptor density (Peroutka and Snyder, 1980a; Crews et al., 1983), while ECT has the opposite effect (Kellar et al., 1981). Chronic antidepressant administration has no effect on serotonin$_1$ ($5HT_1$), muscarinic or dopamine receptors in brain (Sugrue, 1983).

The major current hypothesis of antidepressant action to evolve over the past several years postulates that the therapeutic response to antidepressants is due to a gradual down-regulation of central β-adrenergic receptors (Sulser, 1979). It has been found that chronic, but not acute, treatment with all forms of antidepressants can decrease both the density of central β-adrenergic receptor binding sites (Banerjee et al., 1977; Bergstrom and Kellar, 1979; Scott and Crews, 1983) and the noradrenergic generation of cyclic AMP in brain (Vetulani et al., 1976) with a time course that parallels their clinical response. Other psychotropic agents which have no antidepressant action do not consistently down-regulate central β receptors. Conversely, agents which can precipitate depressive symptoms in certain patients, e.g. reserpine, have been shown to increase the density of central β receptor binding sites and increase the noradrenergic generation of cyclic AMP (Mendels and Frazer, 1974).

Taken together, these findings have lead to the suggestion that endogenous depression may arise from an increase in the sensitivity of the central β-receptor-adenylatecyclase system and that antidepressants may act by decreasing the sensitivity of the β-adrenergic receptor system in brain (Sulser, 1979).

The down-regulation of β-adrenergic receptors by antidepressants is likely due to an increased synaptic concentration of norepinephrine. It has been shown that coadministration of the β receptor antagonist propanolol with antidepressants prevents the β receptor down-regulation (Wolfe et al., 1978; Scott and Crews, 1986) as do prelesions of central adrenergic terminals with 6-hydroxydopamine (Wolfe et al., 1978). In addition, an increase in the amount of norepinephrine released per nerve impulse has been shown in peripheral nerves (Crews and Smith, 1978) and an increased firing rate of the noradrenergic cell bodies of the locus coeruleus (Sverson and Usdin, 1978) has been shown after chronic, but not acute, administration of antidepressants. These increases in release and firing rate appear to be due to desensitization of inhibitory α_2 adrenergic receptors located on adrenergic terminals and on cell bodies of the locus coeruleus (Crews and Smith, 1978; Svenson and Usdin, 1978). Down-regulation of α_2 receptors could be a rate-limiting step in the down-regulation of β receptors by antidepressants (Scott and Crews, 1983).

Chronic treatment with antidepressant drugs has also been found to down-regulate central serotonin$_2$ ($5HT_2$) receptors, and this effect has been implicated in their therapeutic action (Peroutka and Snyder,

1980). In contrast, chronic treatment with electroconvulsive shock increases central $5HT_2$ receptor density (Kellar et al., 1981). Furthermore, the role of the $5HT_2$ receptor in serotonergic transmission is unclear since this receptor has only a very low affinity for serotonin (Peroutka and Snyder, 1979). Although $5HT_2$ receptor down-regulation may be important in the mechanism of action of some antidepressants, it is not a consistent finding with all modes of antidepressant therapy, and, hence, its relevance to the mechanism of action of antidepressants is not clear.

The significance of serotonin in antidepressant action may be related to its interaction with the β-adrenergic receptor system. Several studies indicate that adrenergic and serotonergic nervous systems interact in brain. It has been shown that lesioning central serotonergic terminals with the selective neurotoxin 5,7-dihydroxytryptamine increases the density of central β-adrenergic receptors (Stockmeier et al., 1985). In addition, lesions of central serotonin terminals prevent the down-regulation of central β receptors by several different antidepressants (Brunello et al., 1982). Thus, it is possible that β receptors are, in part, regulated by serotonin, and serotonin may play a permissive role in the down-regulation of β receptors by several antidepressants.

In contrast to serotonin$_2$ and β-adrenergic receptors, a receptor in brain which has received minor attention in studies on antidepressant action is the α_1 adrenergic receptor. When stimulated, this receptor increases membrane phosphatidylinositol turnover (Gonzales and Crews, 1985) and is excitatory for neuronal firing

(Menkes and Aghajanian, 1981). In addition, stimulation of central α_1 receptors in mice appears to mediate a behavioral excitation syndrome (Pichler and Kobinger, 1980). Recent electrophysiologic (Menkes and Aghajan, 1981) and behavioral (Maj et al., 1979) studies suggest that the sensitivity of central α_1 receptors may be enhanced following chronic antidepressant treatment. These findings are in contrast to those of binding studies which have found that the density of central α_1 receptors is not altered by chronic administration of antidepressants (Peroutka and Snyder, 1980).

In contrast to central α_1-receptors, central β-adrenergic receptors have been shown to be inhibitory for neuronal firing (DeMontigny et al., 1980) and are known to decrease in both density and responsiveness following chronic antidepressant therapy. Thus, the enhanced α_1 receptor mediated excitatory response observed after chronic administration of antidepressants may be secondary to a reduction in the inhibitory input of β receptors. This balance between excitatory α_1 and inhibitory β adrenergic input in the brain may be fundamental to the etiology and therapy of endogenous depression. We propose that depression may result from excessive inhibitory β receptor input relative to excitatory α_1 receptor input, and antidepressants may act by restoring the balance between central β and α_1 receptors.

CHAPTER TWO
RAPID DECREASE IN RAT BRAIN β-ADRENERGIC RECEPTOR BINDING DURING COMBINED ANTIDEPRESSANT-α_2 ANTAGONIST TREATMENT

Introduction

The clinical response to tricyclic and monoamine oxidase inhibitor antidepressants requires several weeks to develop (Klein and Davis, 1969; Oswald et al., 1972). During the last several years, studies have found that chronic treatment with antidepressants can decrease both the density of cerebral cortical β-adrenergic receptor binding sites (Banerjee et al., 1977; Bergstrom and Kellar, 1979a) and the noradrenergic generation of cyclic AMP in brain (Vetulani et al., 1976). Decreases in β-adrenergic binding and responsiveness have also been reported following electroconvulsive treatment (ECT) (Bergstrom and Kellar, 1979b). These findings have led to the suggestion that the therapeutic effect of antidepressants is due to a progressive desensitization of central β-adrenergic receptors (Sulser, 1979). This down-regulation of β-receptors is likely to be due to an increased synaptic concentration of norepinephrine (Wolfe et al., 1978). An increase in the amount of norepinephrine released per nerve impulse during chronic, but not acute, antidepressant treatment occurs in peripheral nerves (Crews and Smith, 1978). This increase in release appears to be due to a loss of presynaptic α_2 receptor inhibition of release (Crews and Smith, 1980). Desensitization of α_2

receptors could be a limiting step in increasing the synaptic concentration of norepinephrine. In previous studies, we have shown that the β-adrenergic receptor antagonist phenoxybenzamine (PBZ) accelerates and enhances the desipramine (DMI)-induced β receptor desensitization in rat cerebral cortex (Crews et al., 1981). The purpose of the present study was to investigate the effects of α-adrenergic receptor blockade on antidepressant-induced β receptor desensitization in various regions of the rat brain and to investigate other α-adrenergic antagonists in combination with various other antidepressants.

Methods

Male Sprague-Dawley rats (250-350g) were housed in a well ventilated room with food and water available ad libitum. Lights were on between 800 and 1600 hours. Drugs were administered by i.p. injections usually at about 10:00 a.m. and 6:00 p.m. Animals were sacrificed by decapitation approximately 16 or 24 hours after the last drug treatment, and their brains were rapidly removed and placed in ice-cold saline. The cerebral cortex, including the frontal lobes, mesencephalon, cerebellum and hippocampi were dissected and removed intact. The mean wet weight ± the standard error of the mean for these brain regions was as follows: cerebral cortex, 453 ± 27 mg, n = 16; mesencephalon, 184 ± 9 mg, n = 16; cerebellum 350 ± 11 mg, n = 16; hippocampus 133 ± 7 mg, n = 16. The tissues were homogenized in 5 ml of ice-cold 50 mM Tris (hydroxymethyl) aminomethane, adjusted to pH 7.7 with HCl (Tris-HCl), using a Brinkman Polytron at setting 50 for approximately 30 seconds. The homogenates were centrifuged at 49,000g

for 15 minutes at 4°C, the supernatant discarded, and the pellets re-homogenized in 5 ml of fresh 50 mM Tris-HCl, pH 7.7. After a second centrifugation at 49,000g for 15 minutes, the pellets were homogenized as before in 20 volumes per gram wet weight of tissue.

Determination of [^{3}H]-Dihydroalprenolol ([^{3}H]-DHA) Binding to Brain Region Homogenates

The densities of β-adrenergic receptor sites in all regions studied were measured by a modification of the method of Bylund and Snyder (1976). Briefly, [^{3}H]-DHA binding was determined by incubating 150 μl of tissue homogenate with 850 μl of 50 mM Tris-HCl buffer pH 8.0 containing various concentrations of [^{3}H]-DHA for 20 minutes at 25°C. Total binding was determined in triplicate and nonspecific binding was determined in duplicate in the presence of 5 μM l-alprenolol. Specific binding was calculated as the difference between total and nonspecific binding. Scatchard analyses were done using concentrations of [^{3}H]-DHA ranging from 0.1 nM to 4.0 nM. Samples were incubated for 20 minutes, filtered over Whatman GF/B filters and washed 4 times with 5 ml of ice-cold 50 mM Tris-HCl buffer. The filters were placed in vials containing 8 ml of scintillation fluid and shaken for at least 1 hour. Radioactivity was determined with a Beckman LS 7500 scintillation counter with an efficiency of counting of approximately 35%.

Drugs and Reagents

The following drugs and reagents were used: 1-[propyl-2,3-^{3}H]dihydroalprenolol (specific activity 33 Ci/mmol, radiochemical purity >98%, Amersham Corporation),

l-alprenolol-D-tartrate, pargyline hydrochloride, and yohimbine hydrochloride (Sigma Chemical Co.). The following drugs were generously donated by the companies indicated: desipramine hydrochloride (Merrell, Dow Pharmaceuticals, Inc.), amitriptyline hydrochloride (Merck, Sharp and Dohme Research Lab), iprindole hydrochloride (Wyeth Laboratories), tranylcypromine sulfate and phenoxybenzamine hydrochloride (Smith, Kline and French Labs.), mianserin hydrochloride (Organon, Inc.), dihydroergotamine methane sulfonate (Sandoz Pharmaceuticals), and prazosin hydrochloride (generously donated by Dr. K. Kadzielawa).

All drugs were dissolved in physiological buffered saline except dihydroergotamine and prazosin. The dihydroergotamine was prepared at a concentration of 9.5 mg/ml in 10% (w/v) sucrose and 3% (v/v) dimethylsulfoxide (DMSO). Prazosin was dissolved at a concentration of 2.2 mg/ml in 10% (w/v) sucrose, 17% (v/v) ethanol, and 1% (v/v) DMSO. Animals designated as controls received injections of vehicle which were identical in volume and composition to the solvent injected with the drug in the corresponding test group.

Statistical Analysis

Data are expressed as the mean ± S.E.M. The Student's t-test was used to evaluate the differences between two means for significance. The criterion for significance was $p<0.05$. Scatchard plots were determined by calculating best-fitting lines to the data points by least-squares linear regression analysis. Values for maximum binding, (B_{max}) and apparent affinity (K_d) were determined using the Rosenthal

derivation (Rosenthal, 1967) of Scatchard analysis (Scatchard, 1948). Apparent K_i values were calculated by the formula:

$$K_i = IC_{50}/(1 + \frac{[^3H\text{-DHA}]}{K_d})$$

The IC_{50} (median inhibitory concentration) was calculated by probit analysis (Goldstein, 1964).

Results

Brain Regions

We have previously shown that treatment with DMI alone required at least 6 days to decrease the number of cerebral cortical β-adrenergic receptor sites, whereas treatment with DMI in combination with PBZ decreased the number of β-adrenergic binding sites after only one day of combined DMI/PBZ treatment (Crews et al., 1981). To determine if the rapid desensitization of cortical β-adrenergic receptors was similar in other brain regions, we studied the effects of DMI administration alone or in combination with PBZ on β-adrenergic receptor binding sites in the hippocampus, mesencephalon, and cerebellum. We divided the treatment into two daily doses of DMI (3.75 mg/kg) or DMI/PBZ (3.75 mg/kg each), as opposed to 7.5 mg/kg of each drug given once daily in our previous studies. Under these conditions, we again found a significant decrease in β-adrenergic receptor binding in the cerebral cortex after only one day of combined DMI/PBZ treatment (Figure 1, upper panel). Three days of combined DMI/PBZ treatment further reduced β receptor binding to 67.0 ± 2.5% of control values. Treatment for 7 days with the divided dosage schedule significantly reduced β-adrenergic receptor binding in groups

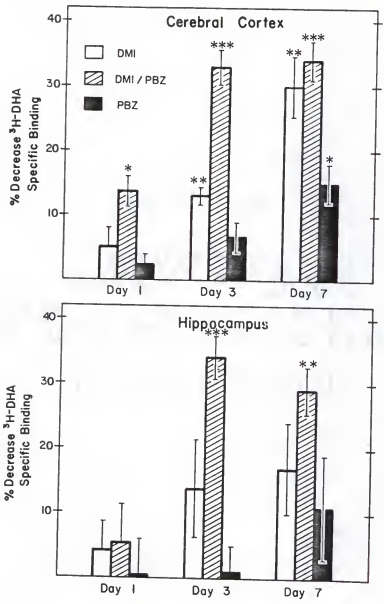

Figure 1. Effects of desipramine (DMI, 3.75 mg/kg, b.i.d.) and phenoxybenzamine (PBZ, 3.75 mg/kg, b.i.d.) administration alone or in combination on the development of cerebral cortical and hippocampal β-adrenergic receptor desensitization. Values are the mean ± S.E.M. of the percent change in the binding of [^{3}H]-DHA to membranes from drug-treated animals compared with vehicle-treated controls. Animals were sacrificed approximately 16 hours after the last dose. Membranes prepared from 6 animals in each group were incubated with 4 nM [^{3}H]-DHA for 20 minutes. Nonspecific binding was determined in the presence of 5 μM alprenolol. Control values were 10.67 ± 0.64 and 4.77 ± 0.48 pmol [^{3}H]-DHA bound/g wet wt. in cerebral cortex and hippocampus, respectively. *p<0.05, **p<0.01, ***p<0.001, when compared to control values.

treated with DMI alone and combination DMI/PBZ. Treatment with PBZ alone for 7 days with this divided schedule caused a slight decrease in β receptor binding sites in the cerebral cortex.

In the hippocampus, no treatment group studied significantly reduced the number of β-adrenergic receptors after one day of treatment (Figure 1, lower panel). After 3 days of treatment, there was a marked reduction in [^{3}H]-DHA binding from a control value of 6.26 ± 0.26 to 4.16 ± 0.19 pmol [^{3}H]-DHA bound/gm wet wt. ($p<0.001$) in the DMI/PBZ group. A similar decrease was found after 7 days of combination treatment. Although there was a trend toward a decrease in the number of β-adrenergic sites in the hippocampus of rats treated with DMI alone, even after 7 days of treatment we did not find a significant change in the number of β-adrenergic binding sites in this brain region. Saturation analysis of hippocampal membranes indicated that the changes in β receptor binding were due to a decrease in the maximum number of binding sites and not a change in affinity for the receptor (Figure 2). Three days of combined DMI/PBZ administration reduced the B_{max} from a control value of 15.2 pmol [^{3}H]-DHA bound/g wet wt. to 9.6 pmol [^{3}H]-DHA bound/g wet wt. Thus, combination DMI/PBZ treatment enhanced the rate of β-adrenergic receptor down-regulation in the hippocampus as well as the cerebral cortex.

Although the changes in the number of β-adrenergic receptor binding sites in the mesencephalon were similar to those in the hippocampus and cerebral cortex, the cerebellum was markedly different. Mesencephalic β receptor binding did not change after one day of treatment in any of the groups studied. Combination treatment

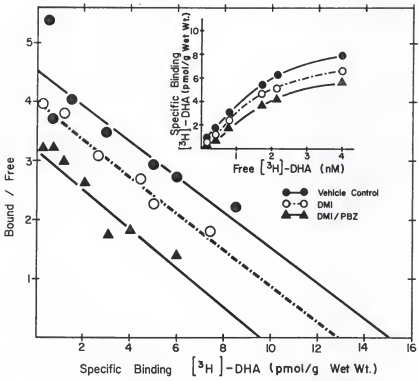

Figure 2. Scatchard analysis of [^{3}H]-DHA binding to hippocampal membranes from rats treated with vehicle, DMI (3.75 mg/kg, b.i.d.), or DMI (3.75 mg/kg, b.i.d.) + (PBZ 3.75 mg/kg, b.i.d.) for three days. Ordinate, bound/free, ratio of specifically bound to free [^{3}H]-DHA. The inset shows the saturation isotherm of [^{3}H]-DHA binding as a function of increasing concentrations of [^{3}H]-DHA.

for 3 and 7 days markedly reduced [^{3}H]-DHA binding in the mesencephalon, whereas DMI or PBZ treatment alonecaused no significant change in this brain region (Figure 3, upper panel). In contrast to the other brain regions studied, treatment with DMI alone or in combination with PBZ did not significantly affect the number of β-adrenergic binding sites in the cerebellum even after 7 days of treatment (Figure 3, lower panel). Thus, PBZ can enhance and accelerate DMI-induced β-adrenergic receptor desensitization in the cerebral cortex, hippocampus, and mesencephalon.

Other Antidepressants

To determine if combined treatment with PBZ enhances the down-regulation of β receptors by various antidepressants other than DMI, we administered PBZ for two days in combination with several chemically different antidepressants. In contrast to DMI, amitriptyline (15 mg/kg/day), a prototype tricyclic which is a potent inhibitor of neuronal serotonin transport (Koe, 1976), did not decrease [^{3}H]-DHA binding after two days of treatment alone or in combination with PBZ (Table 2). In addition, two atypical antidepressants which are not uptake inhibitors, i.e. mianserin and iprindole were studied. Mianserin, a new clinically effective antidepressant which has some α_2 blocking activity on its own (Nickolson et al., 1981) had no effect when administered alone for two days. However, in combination with PBZ it reduced [^{3}H]-DHA binding significantly from controls ($p<0.025$) and animals treated with mianserin alone ($p<0.05$) (Table 2). Iprindole, another clinically effective tricyclic which does not block serotonin or norepinephrine

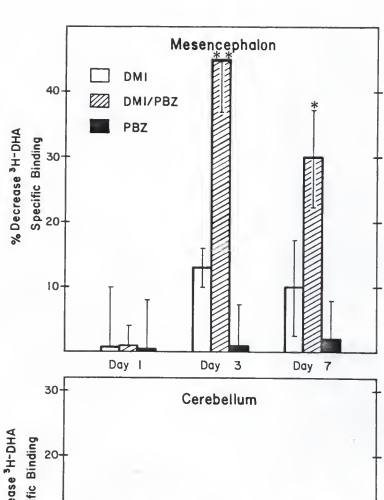

Figure 3. Effects of desipramine (DMI, 3.75 mg/kg, b.i.d.) and phenoxybenzamine (PBZ, 3.75 mg/kg, b.i.d.) administration alone and in combination on the development of mesencephalon and cerebellar β-adrenergic receptor desensitization. Values are the mean ± S.E.M. of the percent change of the binding of [^{3}H]-DHA to membranes from drug-treated animals compared with vehicle-treated controls. Animals were sacrificed approximately 16 hours after the last dose. Membranes were prepared from 6 animals in each group. Mesencephalon membranes were incubated with 0.8 nM [^{3}H]-DHA for 20 minutes. Nonspecific binding was determined in the presence of 5 μM alprenolol. Control mesencephalon values were 1.30 ± 0.09 pmol [^{3}H]-DHA bound/g wet wt. Cerebellar membranes were incubated with 4 nM [^{3}H]-DHA and had a control value of 4.78 ± 0.44 pmol [^{3}H]-DHA bound/g wet wt. *$p<0.05$, **$p<0.01$, when compared to control values.

transport was also effective after two days of treatment only when administered in combination with PBZ. [^{3}H]-DHA binding in animals treated with combination iprindole/PBZ was 9.16 ± 0.39 pmol [^{3}H]-DHA bound/gm wet wt. which is significantly ($p<0.05$) less than binding to membranes from control 10.3 ± 0.2 pmol/g wet wt. and iprindole 10.7 ± 0.4 pmol/g wet wt. treated animals (Table 2). Saturation analysis indicated that the decreases in binding for both mianserin/PBZ and iprindole/PBZ treatment groups were decreases in the number of [^{3}H]-DHA binding sites, i.e. B_{max}, and not changes in affinity, i.e. K_d (data not shown).

Monoamine oxidase inhibitors (MAOI) are known to be effective in the treatment of depression and to reduce the number of β receptor binding sites and the responses of adenylate cyclase to β receptor stimulation during chronic treatment (Sulser, 1979). We administered two chemically different MAOI, i.e. tranylcypromine and pargyline, alone or in combination for two days. Neither drug caused any change in [^{3}H]-DHA binding when administered alone for two days. However, both drugs significantly ($p<0.05$) decreased [^{3}H]-DHA binding when given in combination with PBZ for two days (Table 2). Thus, combined treatment with PBZ and most antidepressants can rapidly down-regulate the β-adrenergic receptor.

TABLE 2: Changes in [^{3}H]-DHA binding after treatment with antidepressants alone or in combination with PBZ[a].

Antidepressant	% Control Values[b] Antidepressant	% Control Values[b] Antidepressant Alone + PBZ[c]
Typical		
DMI (7.5 mg/kg)	95 ± 4	80 ± 3*
Amitriptyline (15 mg/kg)	100 ± 4	97 ± 1
Atypical		
Mianserin (10 mg/kg)	97 ± 6	89 ± 4*
Iprindole (10 mg/kg)	105 ± 5	89 ± 3*
Inhibitor		
Tranylcypromine (10 mg/kg)	97 ± 4	74 ± 4*
Pargyline (10 mg/kg)	98 ± 2	80 ± 3*

a. All drugs given for 2 days i.p. once daily. Six animals were in each group. Animals were sacrificed 24 hours after the last injection. *$p<0.05$ when compared to control values.

b. Control values were determined at 2.9-3.1 nM [^{3}H]-DHA. Control values ranged from 10.25 to 12.38 pmole [^{3}H]-DHA bound/g wet wt. tissue.

c. PBZ (Phenoxybenzamine) was administered at a dose of 7.5 mg/kg.

Other α Antagonists

Since PBZ is known to interact with α_2-adrenergic receptors, α_1-adrenergic receptors, and several other receptor types, we studied the effects of other α-adrenergic blockers in combination with DMI. After two days of treatment with a low dose of yohimbine (3.75 mg/kg b.i.d.) in combination with DMI there was a marked decrease in [^{3}H]-DHA binding (Figure 4). Animals treated with DMI alone or any of the α-antagonists alone for 2 days did not show any significant change in β-adrenergic receptor number. Similar to the findings with DMI/PBZ treatment, Scatchard analysis indicated that the decreases in binding were due to reductions in the density of β-adrenergic receptors, i.e. B_{max} (Figure 5). Combination DMI/yohimbine treatment reduced the B_{max} from a control value of 8.6 pmol/g wet wt. to 5.7 pmol/g wet wt., whereas K_d values were similar, being 0.75 nM and 0.77 nM for control and DMI/yohimbine groups, respectively. In contrast to yohimbine, treatment with a low dose of DHE, 7.5 mg/kg/day, in combination with DMI did not change [^{3}H]-DHA binding after two days of treatment (data not shown). A larger dose of DHE, i.e. 40 mg/kg/day, in combination with DMI, 3.75 mg/kg b.i.d., did reduce β receptor binding after two days of treatment (Figure 4). Prazosin, an α_1 selective drug, was studied at doses of 7.5 and 20 mg/kg/day. Higher doses were limited by the solubility of prazosin. Treatment of rats with either dose of prazosin alone or in combination with DMI for two days did not change β receptor binding (Figure 4). The effectiveness of yohimbine and the lack of effect of prazosin suggest that blockade of the α_2-adrenergic

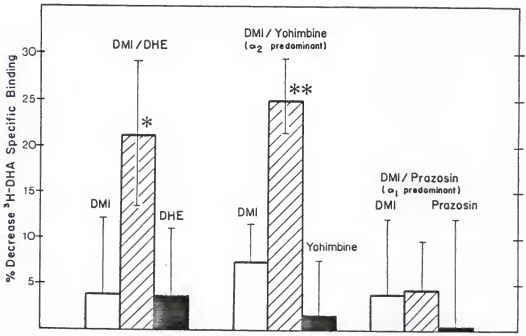

Figure 4. Effects of DMI (3.75 mg/kg, b.i.d.) administered alone or in combination with dihydroergotamine (DHE, 20 mg/kg, b.i.d.), yohimbine (3.75 mg/kg, b.i.d.) or prazosin (10 mg/kg, b.i.d.) on cerebral cortical β-adrenergic receptor desensitization. Drugs were administered alone or in combination for 2 days to four groups of 6 animals each. Membranes were prepared and the binding of [^{3}H]-DHA determined as described in Figure 1. Values are the mean ± S.E.M. of the percent change in [^{3}H]-DHA binding from drug-treated animals compared with vehicle-treated controls. *$p<0.05$, **$p<0.01$, when compared to control values.

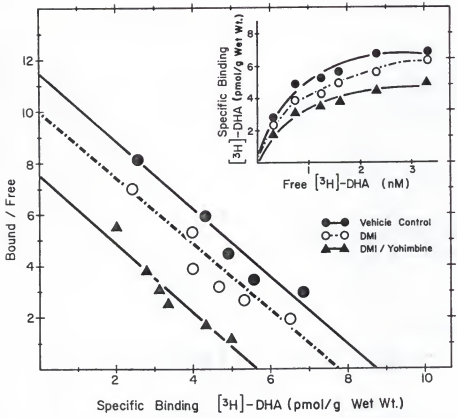

Figure 5. Scatchard analysis of [^{3}H]-DHA binding to cerebral cortical membranes from rats treated with vehicle, DMI (3.75 mg/kg, b.i.d.) or DMI (3.75 mg/kg, b.i.d.) + yohimbine (3.75 mg/kg, b.i.d.) for 2 days. Bound/free, ratio of specifically bound to free [^{3}H]-DHA. The inset shows the saturation isotherm of [^{3}H]-DHA binding as a function of increasing concentration of [^{3}H]-DHA.

receptor is important for the enhancement of the DMI-induced decrease in binding.

The effectiveness of yohimbine prompted a study of amitriptyline in combination with yohimbine, since two days of amitriptyline/PBZ treatment did not reduce [^{3}H]-DHA binding. Treatment with amitriptyline alone required 10 days to reduce [^{3}H]-DHA binding (Figure 6). Combined amitriptyline/yohimbine treatment decreased β-adrenergic receptor binding by 22.5 ± 4.6% ($p<0.05$) after six days of treatment. Similar values were obtained after 10 days of combination amitriptyline/yohimbine treatment. The decreases found with combination treatment after 6 and 10 days were significantly ($p<0.05$) greater than the decrease found after 10 days of treatment with amitriptyline alone. Thus, combinations of α_2 antagonists with several different tricyclic antidepressants can accelerate and enhance the down-regulation of β-adrenergic receptors.

To determine the affinity of the various drugs studied for β-adrenergic receptors, drugs were added in vitro to cerebral cortical membrane preparations alone or in combination. All of the drugs studied had a very low affinity for the β-adrenergic receptor. In fact, only dihydroergotamine had a K_i value below 1 μM (Table 3). Dihydroergotamine was the only drug which reduced [^{3}H]-DHA binding at 0.1 μM. As this concentration is higher than that expected to be in washed membrane preparations, these results suggest that neither DMI nor PBZ interfere with [^{3}H]-DHA binding. This point is supported by the observation that 2 days of dihydroergotamine treatment (40 mg/kg/day) did not significantly alter [^{3}H]-DHA binding (Figure 4).

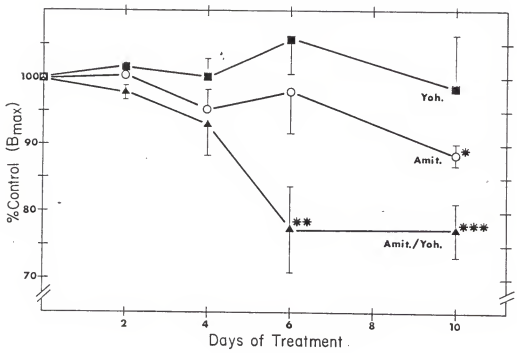

Figure 6. Effects of amitriptyline (Amit., 10 mg/kg, b.i.d.) and yohimbine (yoh., 5 mg/kg, b.i.d.) administration alone or in combination on the development of cerebral cortical β-adrenergic receptor desensitization. Animals were administered vehicle or drug i.p. and [^{3}H]-DHA binding determined, as in Figure 1, 24 hours after the last dose. Values are the mean ± S.E.M. of the percent change in the density of [^{3}H]-DHA binding sites in cerebral cortical membranes from drug-treated animals compared with vehicle-treated controls. Control values were 9.76 ± 1.81 pmol [^{3}H]-DHA bound/g wet wt. *p<0.05, **p<0.01, ***p<0.001, when compared to control values.

TABLE 3: Inhibition of [^{3}H]-DHA binding to cerebral cortical membranes by antidepressants and α-blockers.

	K_i(nM)[a]
Antidepressants:	
Typical -	
Desipramine	3,701 ± 182
Amitriptyline	4,075 ± 317
Atypical -	
Iprindole	29,764 ± 6,801
Mianserin	6,852 ± 1,305
M.A.O. Inhibitors -	
Pargyline	>10^6
Tranylcypromine	11,407 ± 1,808
α-Blockers:	
Phenoxybenzamine	>10^6
Yohimbine	26,267 ± 8,633
Dihydroergotamine	897 ± 89
Prazosin	136,000 ± 12,300

Apparent $K_i = IC_{50}/(1 + [^3H]\text{-DHA}/K_d)$, where K_d ([^{3}H]-DHA) = 1.25 nM as determined in 16 experiments. The median inhibitory concentration (IC_{50}) of each drug was determined by log-probit analysis of the inhibition of [^{3}H]-DHA binding by three or four concentrations of drug ranging from 0.1 to 100 μM. The concentration of [^{3}H]-DHA ranged from 2.89 to 3.38 nM in various experiments. Each value is the mean ± S.E.M. of three determinations.

Discussion

Administration of PBZ in combination with DMI accelerated the decrease in β receptor binding in the cerebral cortex, hippocampus and mesencephalon (Figures 1 and 3). The decreases in binding in cortex and hippocampus are due to a reduced number of β-adrenergic receptors and not a change in affinity (Crews et al., 1981; Figure 2). In the cerebellum, treatment with DMI alone or in combination with PBZ did not change [^{3}H]-DHA binding after even seven days of treatment. Other studies have also reported no change in cerebellar receptor binding during chronic antidepressant treatment (Greenberg and Weiss, 1979). One possible explanation is that the cerebellum contains a high density of β_2-adrenergic receptors on cellular elements such as glia (Minneman et al., 1979) which are not innervated and, therefore, do not change during antidepressant treatment. Our studies using [^{3}H]-DHA do not distinguish between β_1- and β_2-receptor subtypes. However, other studies have shown that DMI administration for 10 days decreases β_1 receptor density in cerebral cortex, with no effect on the density of β_2-receptors (Minneman et al., 1979). It is possible that β_1 receptors in the cerebellum are actually down-regulated, but cannot be detected due to a predominance of β_2 receptors. Whatever the reason for a lack of a detectable decrease in cerebellum, combination treatment with DMI/PBZ accelerated the down-regulation of β-receptors in cerebral cortex, hippocampus and mesencephalon.

The synergism found during combined DMI/PBZ treatment was similar for several other chemically different antidepressants in combination with PBZ (Table 2). Although two days of combined amitriptyline/PBZ

treatment did not reduce [^{3}H]-DHA binding, a longer study of amitriptyline in combination with yohimbine accelerated the down-regulation of β-adrenergic receptors by amitriptyline (Figure 6). PBZ in combination with two atypical antidepressants, mianserin and iprindole, as well as the MAOI, tranylcypromine and pargyline, decreased β receptor density after two days of treatment. These results indicate that PBZ in combination with several different antidepressants accelerates the down-regulation of β-adrenergic receptors.

Since PBZ can interact with receptors other than the α_2-adrenergic receptor, we tested several other α antagonists to confirm our hypothesis that α_2 receptor blockade was important for the synergistic interaction between antidepressants and PBZ. Low doses of yohimbine, a selective α_2 antagonist, enhanced the decrease in β receptor binding by both DMI (Figure 4) and amitriptyline (Figure 6). Recent studies have found that yohimbine in combination with iprindole accelerates the decline in β-receptor binding (Reisine, et al., 1982). In contrast, high doses of prazosin, a selective α_1 antagonist (Starke, 1981), did not enhance DMI-induced down-regulation of β receptors. Although the penetration of drugs into the central nervous system or pharmacokinetic interactions could partially explain these data, it seems likely that blockade of central α_2-adrenergic receptors is the mechanism by which PBZ and yohimbine accelerate the antidepressant induced decrease in β-adrenergic receptors. In support of this are the following: combined administration of DMI/PBZ does not increase the brain levels of DMI (Crews et al., 1981). Typical,

atypical, and MAOI antidepressant-induced desensitization of β receptors is accelerated by PBZ. In all cases studied, we found decreases in the B_{max} suggesting a decrease in the density of β receptors. Antagonists of the α_2 receptors, but not α_1 receptors, are synergistic with antidepressants in decreasing β receptor density. Direct effects due to drug administration are unlikely since antidepressants and α-antagonists have relatively low affinities for the β-adrenergic receptor (Table 3) and would be expected to cause increases in the apparent K_d and not decreases in B_{max}. Taken together, these findings support the hypothesis that α_2-adrenergic antagonists accelerate antidepressant-induced desensitization of β-adrenergic receptors by blocking central α_2-receptors.

The down-regulation of central β receptors during chronic antidepressant treatment is likely to be due to increases in the synaptic concentration of norepinephrine, since destruction of the ascending noradrenergic nerves from the locus coeruleus or administration of propranolol, a β receptor antagonist, can prevent antidepressant-induced down-regulation of β receptors (Wolfe et al., 1978). Studies of norepinephrine release in both the peripheral (Crews and Smith, 1978) and central nervous system (Schoffelmeer and Mulder, 1982) have found that chronic antidepressant treatment increases norepinephrine release. Numerous studies have shown that α_2 receptor antagonists increase norepinephrine release (Starke, 1981). Furthermore, α_2 antagonists block the feedback inhibition of locus coeruleus nerve firing which occurs during acute, but not chronic, antidepressant treatment (Svensson and Usdin, 1978). It has been

suggested that supersensitive α_2 adrenergic receptors underlie the pathology of depression (Garcia-Sevilla et al., 1981), and that desensitization of α_2 receptors are the therapeutically significant action of antidepressants (Crews and Smith, 1980). However, α_2 antagonists by themselves are not known to be antidepressants and do not rapidly down-regulate cerebral cortical β-receptors. In any case, the combination of antidepressant and α_2 antagonists are likely to acutely increase the synaptic concentration of norepinephrine and thereby accelerate the down-regulation of central β adrenergic receptors.

The rapid desensitization of β-receptors during administration of antidepressants and α-adrenergic antagonists has several important implications. Since post-synaptic β-receptors are thought to be directly involved in the therapeutic actions of antidepressants (Sulser, 1979), combined administration of α_2-antagonists with antidepressants may provide a rapid onset therapy. In addition, the greater reduction in β-receptor density produced by combined antidepressant-α_2-antagonist treatment may enhance the effects of antidepressants in previously nonresponsive patients. Although the hypotensive actions of α-antagonists and antidepressants will complicate clinical trials, the observation that combination treatment enhances β receptor desensitization will allow an indirect clinical test of the β receptor hypothesis. Finally, the synergism of α_2-antagonists with clinically effective antidepressants could provide a rapid screen for new antidepressant compounds.

CHAPTER THREE
RAPID DOWN-REGULATION OF SEROTONIN$_2$ RECEPTOR BINDING DURING COMBINED TRICYCLIC ANTIDEPRESSANT-α_2 ANTAGONIST ADMINISTRATION

Introduction

The biogenic amines norepinephrine and serotonin have been implicated in the etiology of endogenous depression. Although most research has focused on the role of either central noradrenergic or serotonergic transmission in depression, it is possible that it is a defect in the interaction of these two systems that underlies the pathology of depression. Considerable evidence suggests that the tricyclic antidepressants down-regulate central β-adrenergic receptors with a time course that corresponds to the onset of their clinical actions (Banerjee, Kung, Riggi and Chanda, 1979; Crews, Paul and Goodwin, 1981). Recent studies have found that chronic, but not acute, treatment with several different antidepressants also decreases cerebral cortical serotonin$_2$ ($5HT_2$) receptor density (Peroutka and Snyder, 1980a). This change in $5HT_2$ receptor density could be an important therapeutic action of antidepressants.

In addition to the $5HT_2$-receptor and β-adrenergic receptor, considerable attention has been focused on the inhibitory α_2-adrenergic receptor. Clinical studies have shown that platelets from depressed patients have increased numbers of α_2-adrenergic receptors (Garcia-Sevilla, Zis, Hollingsworth, Greden and Smith,

1981). In addition, chronic antidepressant treatment of rats has been shown to decrease presynaptic α_2 receptor inhibition of norepinephrine release (Crews and Smith, 1978) and locus coeruleus firing (Svensson and Usdin, 1978). Thus, α_2 adrenergic receptors may also be involved in the therapeutic effects of antidepressants.

We have found that the ability of antidepressants to down-regulate central β-adrenergic receptors is enhanced and accelerated when antidepressants are administered in combination with α_2 antagonists (Crews et al., 1981; Scott and Crews, 1983). Since $5HT_2$ receptors are also implicated in the therapeutic actions of antidepressants, we decided to study the effects of antidepressants alone or in combination with α_2 antagonists on the density of cerebral cortical $5HT_2$ receptors. We have found that α_2 antagonists do enhance and accelerate tricyclic antidepressant-induced decreases in $5HT_2$ receptor density. Thus, it is possible that the α_2 receptor represents an important link between the activity of central noradrenergic and serotonergic systems.

Methods

Animal Treatment and Tissue Preparation

Male Sprague-Dawley rats (250-350g) were housed in a well ventilated room with food and water available ad libitum. Lights were on between 800 and 1600 hours. Drugs were administered by i.p. injection usually at about 9:00 a.m. and 6:00 p.m. Animals were sacrificed by decapitation 24 hours after the last drug treatment, their brains were rapidly removed and placed in ice-cold phosphate buffered saline, pH 7.4. The cerebral cortex with its frontal lobes

was dissected, weighed (mean wet weight ± S.E.M. of 0.496 ± 0.010 g, n = 15), and placed in 5 ml of ice-cold 50 mM Tris(hydroxymethyl)-aminomethane (Tris), adjusted to pH 7.5 with HCl. Samples were then homogenized with a Brinkman Polytron at setting 50 for approximately 30 seconds. The homogenates were centrifuged at 49,000 g for 15 minutes at 4°C, the supernatant discarded, and the pellets rehomogenized in 5 ml of fresh 50 mM Tris. After a second centrifugation at 49,000 g for 15 minutes, the samples were homogenized as before in 20 ml of 50 mM Tris per gram wet weight of tissue.

Determination of [^{3}H]-Spiperone Binding to Cerebral Cortex Homogenates

In this paper, we define $5HT_2$ receptor binding as the calculated specific binding obtained using [^{3}H]-spiperone for total binding and [^{3}H]-spiperone in the presence of 25 μM ketanserin, 25 μM mianserin or 30 μM d-lysergic acid as nonspecific binding. The drugs used are specified in the figure legends. Approximately 6 hours elapsed between the sacrifice of animals and binding assays, during which the tissue was always ice-cold. [^{3}H]-Spiperone binding was determined by incubating 150 μl of tissue homogenate with 1850 μl of 50 mM Tris buffer, pH 6.8-7.0 containing approximately 1.5 nM [^{3}H]-spiperone (21 Ci mmole^{-1}, Amersham) for 15 minutes at 37°C. In most assays, total binding was determined in triplicate, and nonspecific binding was determined in duplicate in the presence of 25 μM ketanserin tartrate. It is essential that the pH of the incubation solution be between 6.8 and 7.0 to prevent the ketanserin from forming a barely noticeable film-precipitate at the meniscus. When mianserin or d-lysergic acid

were used as the nonspecific ligand, [^{3}H]-spiperone binding was determined by incubating 150 μl of tissue homogenate with 850 μl of 50 mM Tris buffer, pH 7.4 containing approximately 0.5 nM [^{3}H]-spiperone for 15 minutes at 37°C. Preliminary studies indicated no difference in binding of [^{3}H]-spiperone between pH 7.0 and pH 7.5. After a 15 minute incubation, samples were filtered through Whatman GF/B filters in all assays and were washed 4 times with 5 ml of ice-cold Tris buffer. The filters were then placed in vials containing 7 ml of Liquiscint scintillation fluid (National Diagnostics) and shaken for at least 1 hour. Radioactivity was determined with a Beckman LS 7500 scintillation counter with a counting efficiency of approximately 35%. Saturation analyses were done using concentrations of [^{3}H]-spiperone ranging from 0.5 to 4.0 nM. Values for maximum binding, i.e. B_{max}, and apparent affinity, i.e. K_d, were determined using the Rosenthal derivation (Rosenthal, 1967) of Scatchard analysis (Scatchard, 1948). Scatchard plots were obtained by calculating the best fitting lines to the data points by least-squares linear regression analysis.

Determination of Drug K_d Values for $5HT_2$ and α_2 Binding Sites in Cerebral Cortex Homogenates

Drugs in concentrations ranging from 0.01 to 100 μM were incubated with [^{3}H]-spiperone or [^{3}H]-p-aminoclonidine to determine their apparent inhibition constants (K_i) for the $5HT_2$ and α_2 receptors, respectively. Cerebral cortical homogenates were prepared from untreated rats as described above. [^{3}H]-spiperone binding in the presence of drug was determined using approximately 1.5 nM [^{3}H]-spiperone and 25 μM ketanserin to measure nonspecific binding.

[^{3}H]-p-aminoclonidine binding was determined by incubating 150 μl of tissue homogenate with 850 μl of 50 mM Tris HCl buffer, pH 7.5, containing approximately 1.5 nM [^{3}H]-p-aminoclonidine (40.5 Ci mmole-^{1}, N.E.N.) and various concentrations of drugs for 30 minutes at 25°C. Total binding was determined in triplicate and nonspecific binding was determined in duplicate in the presence of 1.0 μM phentolamine-mesylate as described by Maggi, U'Prichard, and Enna (1980). From the percent inhibition of [^{3}H]-ligand binding by 3 or 4 concentrations of drug, a competition curve was constructed and the K_i determined by log-probit analysis (Goldstein, 1964).

Statistical Analysis

Data are expressed as the mean ± S.E.M. The Student's t-test was used to evaluate the differences between two means for significance. The criterion for significance was $P<0.05$.

Drugs

The following drugs were kindly donated by the companies indicated: desipramine hydrochloride (Merrell, Dow Pharmaceuticals, Inc.), amitriptyline hydrochloride (Merck, Sharp and Dohme Research Lab), iprindole hydrochloride (Wyeth Laboratories), tranylcypromine sulfate and phenoxybenzamine hydrochloride (Smith, Kline and French Labs.), mianserin hydrochloride (Organon, Inc.), dihydroergotamine methane sulfonate (Sandoz Pharmaceuticals), ketanserin tartrate (Janssen Pharmaceuticals), prazosin hydrochloride and phentolamine mesylate (generously donated by Dr. K. Kadzielawa). Yohimbine

hydrochloride and d-lysergic acid were purchased from Sigma Chemical Co.

All drugs were dissolved in physiologic buffered saline, except those of the DMI/dihydroergotamine and DMI/prazosin experiments, where the drugs were dissolved in 10% (w/v) sucrose solutions. Dihydroergotamine required 3% (v/v) dimethylsulfoxide (DMSO) for its dissolution, and prazosin required 1% (v/v) DMSO and 17% (v/v) ethanol for its dissolution. All drug solutions were at near neutral pH when administered. Control animals received i.p. injections of vehicle which were identical in volume and composition to the solvents of the drugs in the test groups.

Results

Effects of Amitriptyline and Yohimbine Administered Alone or in Combination on Cerebral Cortical $5HT_2$ Receptor Binding

To determine the effects of α_2 receptor blockade on antidepressant-induced desensitization of $5HT_2$ receptors, we administered amitriptyline alone or in combination with yohimbine, an α_2 antagonist. Treatment with amitriptyline (10 mg/kg, b.i.d.) or yohimbine (5 mg/kg, b.i.d.) alone for two, four or six days did not alter $5HT_2$ receptor binding. However, $5HT_2$ receptor binding in the group treated with a combination of amitriptyline/ yohimbine for four days was decreased 22.1 ± 1.3% ($P<0.05$) from controls and 17.0 ± 1.4% ($P<0.001$) from the amitriptyline alone group (Figure 7). After six days of treatment, receptor binding was similar to that observed with four days of treatment. Saturation analysis of membranes prepared from animals treated for four and six days (Figure 8) indicated that

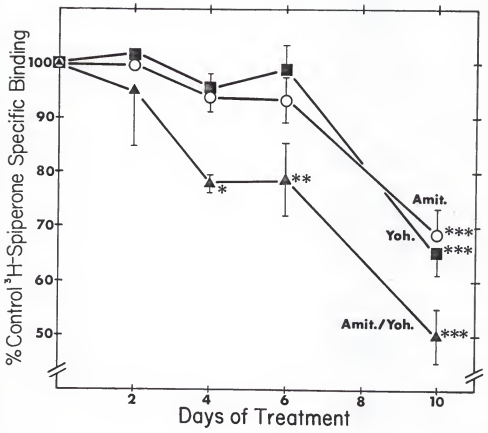

Figure 7. Effects of amitriptyline (Amit., 10 mg/kg, b.i.d.) and yohimbine (Yoh. 5 mg/kg, b.i.d.) administered alone or in combination on the development of cerebral cortical $5HT_2$ receptor desensitization. Values are the mean ± S.E.M. of the percent change of specific binding of [^{3}H]-spiperone to cerebral cortical membranes from drug-treated animals compared with vehicle-treated controls. Each group consisted of 6-12 animals. For each point, specific binding was determined using a single concentration of [^{3}H]-spiperone from 0.80 to 1.5 nm as described in the methods. Nonspecific binding was determined in the presence of 25 μM ketanserin. Control values ranged from 3.18 to 9.27 pmol [^{3}H]-spiperone bound per g wet weight of tissue over this concentration range. *P<0.05; **P<0.01; ***P<0.001 when compared to control values.

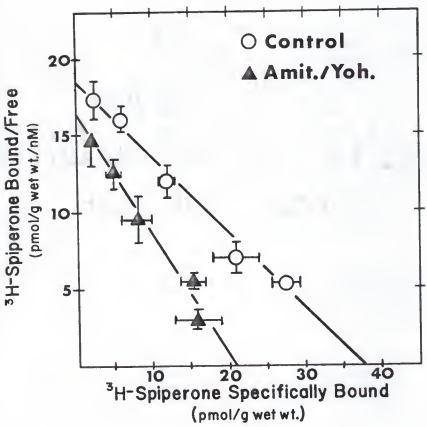

Figure 8. Scatchard analyses of specific [^{3}H]-spiperone binding to cerebral cortical membrane preparations from rats treated with vehicle or amitriptyline in combination with yohimbine (10 mg/kg, b.i.d. and 5 mg/kg, b.i.d., respectively) for 6 days. Values are the mean ± S.E.M. of separate Scatchard analyses on the membranes from 6 animals. The concentrations of [^{3}H]-spiperone used ranged from 0.2 to 5.5 nM. Nonspecific binding was determined in the presence of 25 μM ketanserin.

the decrease in binding with a combination of amitriptyline/yohimbine was due to a decrease in receptor density, i.e. B_{max}. Combination treatment for 6 days decreased the B_{max} from a control value of 37.5 ± 3.9 pmol/g wet wt. to 24.7 ± 1.35 pmol/g wet wt. ($P<0.001$). In addition, we found a slight decrease in the K_d for [^{3}H]-spiperone in the combination group relative to controls. Administration for 10 days decreased $5HT_2$ receptor binding in all three drug-treated groups. When compared to control values, 10 days of yohimbine treatment diminished $5HT_2$ receptor binding by 34.2 ± 4.2% ($P<0.001$), and amitriptyline treatment by 32.0 ± 4.4% ($P<0.001$). Combination treatment reduced binding by 42.4 ± 4.7% ($P<0.001$) from control values. Saturation analysis indicated that both amitriptyline and amitriptyline/yohimbine decreased the maximum binding, whereas yohimbine treatment showed no change in maximum binding (Figure 9). However, in the group treated with yohimbine alone for 10 days there was an increase in the K_d for [^{3}H]-spiperone from a control value of 1.38 ± 0.08 nM to 2.18 ± 0.28 ($P<0.01$). No significant changes in K_d were observed in membranes prepared from rats treated with amitriptyline alone or in combination with yohimbine for 10 days.

Effects of Antidepressants and Phenoxybenzamine Administered Alone or in Combination on Cerebral Cortical $5HT_2$ Receptor Binding

In previous studies, we have shown that phenoxybenzamine (PBZ), an irreversible β-adrenergic receptor antagonist is more active than yohimbine in accelerating and enhancing the desensitization of β-adrenergic receptors by a variety of antidepressants (Scott and Crews, 1983). These findings prompted us to study the effects of PBZ

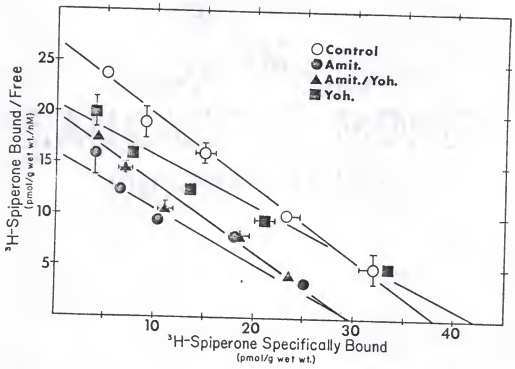

Figure 9. Scatchard analyses of specific [^{3}H]-spiperone binding to cerebral cortical membrane preparations from rats treated with vehicle, amitriptyline (10 mg/kg/day), yohimbine (5 mg/kg/day), or amitriptyline in combination with yohimbine (10 mg/kg, b.i.d. and 5 mg/kg, b.i.d., respectively) for 10 days. Values are the mean ± S.E.M. of separate Scatchard analyses on membranes from 6 animals. [^{3}H]-Spiperone concentrations ranged from 0.2 to 5.8 nM, and nonspecific binding was determined in the presence of 25 μM ketanserin.

in combination with amitriptyline and desipramine (DMI), both prototype tricyclic antidepressants, as well as in combination with two atypical antidepressants, iprindole and mianserin, on $5HT_2$ receptor desensitization. Amitriptyline (15 mg/kg/day) administered in combination with PBZ (7.5 mg/kg/day) for 2 days did not change $5HT_2$ receptor binding (data not shown). Four days of treatment with a higher dose of amitriptyline (25 mg/kg/day) had no significant effect on $5HT_2$ receptor binding. However, combination amitriptyline/PBZ and PBZ alone for four days reduced binding from a control value of 7.25 ± 0.41 to 4.32 ± 0.24 ($P<0.001$) and 4.85 ± 0.40 pmol/g wet wt. ($P<0.01$), respectively (Figure 10). Three days of treatment with a much lower dose of DMI (7.5 mg/kg/day) also had no effect on $5HT_2$ binding. However, combination DMI/PBZ markedly reduced $5HT_2$ receptor binding (Figure 10). As before, PBZ (7.5 mg/kg/day) by itself reduced $5HT_2$ receptor binding. Similar to the yohimbine treatment, Scatchard analysis indicated that three days of PBZ treatment markedly increased the K_d for [^{3}H]-spiperone (Figure 11). In contrast, the density of $5HT_2$ receptors, i.e. B_{max}, was decreased in the group treated with both DMI/PBZ.

The two atypical antidepressants iprindole (10 mg/kg/day) and mianserin (15 mg/kg/day) were administered alone and in combination with PBZ (7.5 mg/kg/day) for 2 days. In contrast to three days of treatment, two days of PBZ administration did not significantly change $5HT_2$ binding. Similarly, two days of treatment with iprindole alone

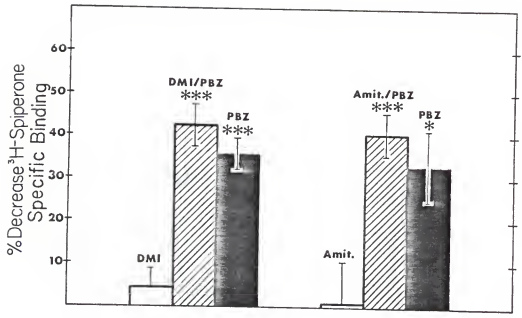

Figure 10. Effects of desipramine (DMI, 7.5 mg/kg/day) and phenoxybenzamine (PBZ, 7.5 mg/kg/day) administered alone or in combination for 3 days or amitriptyline (Amit., 25 mg/kg/day) and PBZ (7.5 mg/kg/day) administered alone or in combination for 4 days on the development cerebral cortical $5HT_2$ receptor desensitization. Values are the mean ± S.E.M. of the percent change in the density of [^{3}H]-spiperone binding sites in cerebral cortical membranes from drug-treated animals compared with vehicle-treated controls. Nonspecific binding was determined in the presence of 25 μM mianserin in the DMI/PBZ experiments and 25 μM ketanserin in the amitriptyline/PBZ experiments. Each group consisted of 6 animals. The control value in the DMI/PBZ experiment was 9.2 pmol [^{3}H]-spiperone bound per g wet weight tissue in the presence of 0.6 nM [^{3}H]-spiperone. The control value in the amitriptyline/PBZ experiment was 9.5 pmol [^{3}H]-spiperone bound per g wet weight tissue in the presence of 1.3 nM [^{3}H]-spiperone. *P<0.05 and ***P<0.001 when compared to control values.

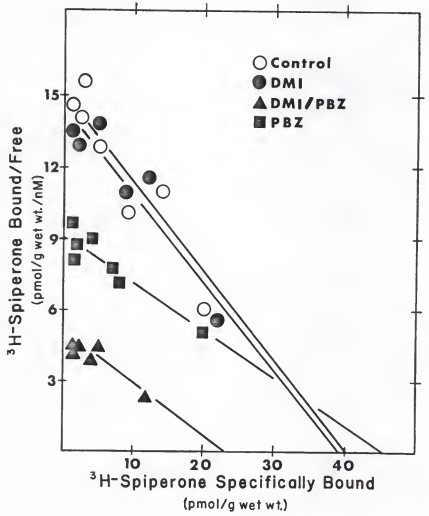

Figure 11. Scatchard analysis of specific [^{3}H]-spiperone binding to cerebral cortical membrane preparations from rats treated with vehicle, desipramine (DMI, 7.5 mg/kg/day), phenoxybenzamine (PBZ, 7.5 mg/kg/day), or combination DMI and PBZ (each 7.5 mg/kg/day) for 3 days. The concentrations of [^{3}H]-spiperone used ranged from 0.06 to 4.3 nM, and nonspecific binding was determined in the presence of 25 μM mianserin.

did not significantly alter $5HT_2$ binding (Figure 12). Treatment with mianserin alone reduced $5HT_2$ binding from a control value of 4.95 ± 0.38 pmol/g wet wt. to 4.27 ± 0.19 pmol/g wet wt. ($P<0.01$). However, combinations of either iprindole or mianserin with PBZ markedly reduced $5HT_2$ receptor binding to 3.45 ± 0.23 pmol/g wet wt. ($P<0.01$) and to 2.28 ± 0.25 pmol/g wet wt. ($P<0.001$), respectively.

Effects of Desipramine and Various Alpha Antagonists Administered Alone or in Combination on Cerebral Cortical $5HT_2$ Receptor Binding

To investigate the effects of other α antagonists alone or in combination with antidepressants, we administered DMI in combination with prazosin, a selective α_1-adrenergic antagonist, and dihydroergotamine (DHE), an α antagonist which interacts with both α_1 and α_2 receptors as well as several other receptor sites (Figure 13). DMI (7.5 mg/kg/day) administered alone or in combination with a high dose of prazosin (20 mg/kg/day) for 2 days had no significant effect on $5HT_2$ receptor binding. Administration of DHE (37.5 mg/kg/day) alone for 2 days caused a slight reduction in $5HT_2$ receptor binding. However, administration of DMI (7.5 mg/kg/day) in combination with DHE (37.5 mg/kg/day) for 2 days markedly reduced $5HT_2$ receptor binding (Figure 13).

Antidepressant and α Antagonist Affinities for $5HT_2$ and α_2 Receptors

Since a knowledge of drug affinity for $5HT_2$ and α_2 receptors is important for interpreting the results of combination therapy, we determined the apparent K_i of the drugs studied for both receptors.

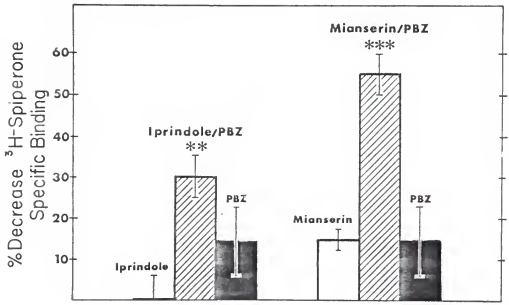

Figure 12. Effects of iprindole (10 mg/kg/day) and mianserin (15 mg/kg/day) administered alone or in combination with phenoxybenzamine (PBZ, 7.5 mg/kg/day) for 2 days on cerebral cortical $5HT_2$ receptor density. Values are the mean ± S.E.M. of the percent change in the density of [^{3}H]-spiperone binding sites in cerebral cortical membranes from drug-treated animals compared with vehicle-treated controls. Each group consisted of 6 animals. The control value was 3.3 pmole [^{3}H]-spiperone bound per g wet weight of tissue in the presence of 0.7 nM [^{3}H]-spiperone. Nonspecific binding was determined in the presence of 25 μM ketanserin. **P<0.01 and ***P<0.001 when compared to control values.

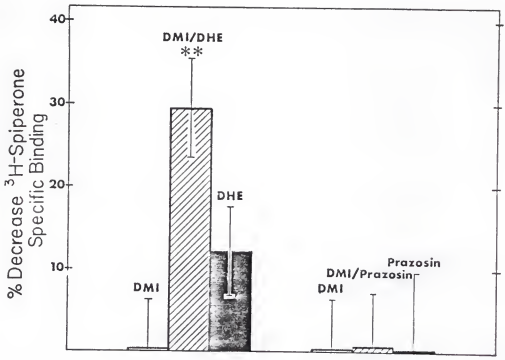

Figure 13. Effects of desipramine (DMI, 7.5 mg/kg/day), dihydroergotamine (DHE, 37.5 mg/kg/day), and prazosin (20 mg/kg/day) administered alone or in combination for 2 days on cerebral cortical $5HT_2$ receptor desensitization. Values are the mean ± S.E.M. of the per cent change in the density of [^{3}H]-spiperone binding sites in cerebral cortical membranes from drug-treated animals compared with vehicle-treated controls. There were 6 animals in each group. The control value was 4.6 pmole [^{3}H]-spiperone bound per g wet weight tissue in the presence of 0.5 nM [^{3}H]-spiperone. Nonspecific binding was determined in the presence of 30 μM d-lysergic acid. **P<0.01 when compared to control values.

When compared to the other antidepressants studied, mianserin had the lowest apparent K_i for both $5HT_2$ and α_2 receptors (Table 4). Amitriptyline had a relatively low apparent K_i for the $5HT_2$ receptor. The other antipressants had relatively high apparent K_i values, i.e. low affinities for both receptors.

The α blockers studied varied greatly in their apparent K_i for the α_2 adrenergic receptor and the $5HT_2$ receptor. Dihydroergotamine had a very high affinity for both $5HT_2$ and α_2 receptors, whereas prazosin had a very low affinity for both receptors. Yohimbine had a low apparent K_i for the α_2 receptor as expected and phenoxybenzamine was found to have a surprisingly low apparent K_i for the $5HT_2$ receptor. Thus, both antidepressants and α antagonists have several fold differences in their relative affinites for $5HT_2$ and α_2 receptors.

Discussion

Our results (Figure 7 and 9) confirm earlier reports that chronic, but not acute, administration of amitriptyline reduces the density of $5HT_2$ receptors in rat cerebral cortex (Peroutka and Snyder, 1980b). We have found that this reduction is accelerated with coadministration of the α_2 antagonist yohimbine. Combined administration of amitriptyline and yohimbine reduced $5HT_2$ receptor binding after only 4 and 6 days of treatment. Scatchard analysis (Figure 8) indicated that this was due to a decrease in the density of $5HT_2$ receptors, i.e. B_{max}, and not a change in affinity. After 10 days of treatment, the B_{max} for [^{3}H]-spiperone was decreased for amitriptyline alone and combination treatment groups. However, 10

Table 4: Inhibition of [^{3}H]-Spiperone and [^{3}H]-p-Aminoclonidine Binding to Cerebral Cortical Membranes by Antidepressants and α-Blockers.

Drugs	[^{3}H]-Spiperone K_i(nM)[a]	[^{3}H]-PAC K_i(nM)[b]
Antidepressants:		
Typical-		
Desipramine	632 ± 110	11,238 ± 609
Amitriptyline	56.1 ± 2.0	1,193 ± 90
Atypical-		
Iprindole	1,476 ± 130	84,589 ± 14,638
Mianserin	33.3 ± 3.4	64.3 ± 3.1
α-Blockers:		
Phenoxybenzamine	78.8 ± 9.2	3,370 ± 61
Yohimbine	1,766 ± 78	132 ± 25
Dihydroergotamine	0.163 ± 0.054	0.009 ± 0.002
Prazosin	20,486 ± 8981	2,632 ± 20

a. Apparent $K_i = IC_{50}/(1+[^3H\text{-Spiperone}]/K_d)$, where K_d(^{3}H-Spiperone) = 1.0 nM. The median inhibitory concentration (IC_{50}) of each drug was determined by log-probit analysis of the inhibition of [^{3}H]-Spiperone binding by 4 or 5 concentrations of drugs ranging from 0.01 μM to 100.0 μM. The concentration of [^{3}H]-Spiperone was 1.69 nM. Each value is the mean ± S.E. of three determinations.

b. Apparent K_i determined as in (a), where K_d(^{3}H-PAC) = 1.6 nM. The concentration of [^{3}H]-PAC was 0.92 nM. Each value is the mean ± S.E. of three determinations.

days of treatment with yohimbine alone reduced 1 point binding by raising the apparent K_d for [^{3}H]-spiperone. This effect may have been due to residual yohimbine in the membrane preparation which competitively inhibited [^{3}H]-spiperone binding to $5HT_2$ receptors even though the apparent affinity of yohimbine for the $5HT_2$ receptor is relatively low (Table 4). Thus, approximately 10 days of treatment were required for amitriptyline alone to reduce $5HT_2$ receptor density, whereas amitriptyline in combination with yohimbine induced decreases in cerebral cortical $5HT_2$ receptor density after only 4 days of treatment.

The reductions in the maximum binding of [^{3}H]-spiperone which we observed after the acute administration of PBZ in combination with the prototype tricyclic antidepressants DMI and amitriptyline, as well as in combination with the atypical antidepressants iprindole and mianserin, suggest that α receptor blockade in combination with antidepressants causes a rapid down-regulation of $5HT_2$ receptors. Since this rapid down-regulation of $5HT_2$ receptors occurs with combinations of antidepressants and yohimbine, an α_2 antagonist, but not prazosin, an α_2 antagonist, it appears that blockade of the α_2 receptor is important for the accelerated down-regulation of cerebral cortical $5HT_2$ receptors by antidepressants. Recent studies by Blackshear and Sanders-Bush (1982) have shown that a single dose of mianserin free base (10 mg/kg) as well as 14 days of treatment with mianserin can reduce $5HT_2$ receptor binding in the frontal cortex by approximately 50%. In our studies, 15 mg/kg mianserin-HCl treatment for two days reduced $5HT_2$ receptor binding 13.8 ± 3.4% ($P<0.01$) in the

entire cerebral cortex including the frontal lobes. The reasons for these differences are not clear. There was no trend toward a decrease in $5HT_2$ receptor binding after two days of treatment with DMI, amitriptyline or iprindole. Amitriptyline required at least one week to reduce $5HT_2$ binding (Figure 7, Perouka and Snyder, 1980b). DMI, amitriptyline, and iprindole (10 mg/kg/day) all reduce $5HT_2$ receptor density after 21 days of administration alone (Perouka and Snyder, 1980a). Thus, α_2 antagonists appear to accelerate the down-regulation of cortical $5HT_2$ receptors induced by DMI, amitriptyline, iprindole and possibly mianserin.

Significant decreases in [^{3}H]-spiperone binding were also observed when PBZ was administered alone for 3 or 4 days. Similar to the 10 day treatment with yohimbine, the decrease with 3 days of PBZ treatment was due to an increased apparent K_d for [^{3}H]-spiperone (Figure 5). PBZ has a high affinity for the $5HT_2$ receptor (Table 4) and could compete for [^{3}H]-spiperone binding. This is likely to be the reason for the decrease in [^{3}H]-spiperone binding after 3 or 4 days of PBZ treatment. Rapid decreases in $5HT_2$ receptor B_{max} occurred only during combination treatment of PBZ and DMI.

The accelerated down-regulation of $5HT_2$ receptor binding with combined administration of antidepressants and α_2 antagonists could be due to pharmacodynamic or pharmacokinetic interactions. Pharmacokinetic interactions are unlikely for the following three reasons: combined administration of DMI and PBZ does not increase the brain levels of DMI (Crews et al., 1981); Scatchard analyses have shown decreases in the B_{max} with combination treatment suggesting

decreases in $5HT_2$ receptor density; antagonists of α_2 receptors, but not α_1 receptors, are synergistic with antidepressants in decreasing $5HT_2$ receptor density. Direct effects due to drug administration are unlikely since antidepressants interact with $5HT_2$ receptors immediately, yet require chronic administration to decrease $5HT_2$ receptor density. In addition, direct effects of antidepressants and α_2 antagonists would be expected to cause increases in the apparent Kd for [^{3}H]-spiperone and not decreases in B_{max}. It appears, therefore, that the accelerated $5HT_2$ receptor down-regulation with coadministration of antidepressants with α_2 antagonists is due to pharmacodynamic interactions.

There are at least three mechanisms by which α_2 antagonists could accelerate the antidepressant induced down-regulation of cortical $5HT_2$ receptors. One possible mechanism involves interactions between postsynaptic β, α_2 and $5HT_2$ receptors. Studies in rat cerebral cortex slices have shown that β receptor stimulation increases α_2 receptor binding (Maggi et al., 1980). Since many α_2 receptors in brain are located postsynaptically (U'Pritchard, Bechtel, Rouot and Snyder, 1979), it is possible that postsynaptic β, α_2 and $5HT_2$ receptors interact such that the rapid down-regulation of β receptors during combined antidepressant/α antagonist treatment (Scott and Crews, 1983) may cause a rapid decrease in $5HT_2$ receptor sites. Thus, direct receptor interactions represent one possible mechanism by which combination antidepressant-α_2 antagonist can rapidly down-regulate $5HT_2$ binding sites.

Whatever the mechanism, the ability of α_2 antagonists to accelerate $5HT_2$ receptor down-regulation by antidepressants has several important implications. Since the reduction of postsynaptic biogenic amine receptor density may be directly involved in the therapeutic actions of antidepressants (Sulser, 1979; Peroutka and Snyder, 1980a,b), combined administration of α_2-antagonists with antidepressants may provide a rapid onset therapy. Although the hypotensive actions of α-antagonists and antidepressants will complicate clinical trials, the observation that combination treatment accelerates 5HT receptor desensitization will allow an indirect clinical test of the biogenic amine receptor hypothesis of depression. Finally, the synergism of α_2-antagonists with clinically effective antidepressants could provide a rapid screen for new antidepressant compounds.

CHAPTER FOUR
STIMULATION OF β-ADRENERGIC RECEPTORS INCREASES SEROTONIN RECEPTOR DENSITY IN RAT CEREBRAL CORTEX SLICES

Introduction

Central noradrenergic and serotonergic pathways appear to modulate and balance each other. The interaction of norepinephrine and serotonin plays a role in the regulation of mood (Schildkraut, 1978; Murphy et al., 1978; Benarroch et al., 1981), blood pressure (Baum and Becker, 1982), pituitary hormone release (Jones et al., 1976), sleep patterns (Morgane, 1981), and many other physiological and behavioral processes. Recent studies have found that the administration of β-adrenergic agonists to rats enhances behavioral responses to serotonergic stimulation (Ortmann et al., 1981; Cowen et al., 1982). These serotonin-related behaviors, e.g. head twitching, resting tremor, forepaw treading, and hind-limb abduction, appear to be mediated by central serotonin$_2$ ($5HT_2$) receptors (Peroutka et al., 1981). In the present study, we provide evidence for an interaction between β-adrenergic and serotonergic$_2$ receptor binding sites which may account for the serotonergic hyperactivity induced by β agonists. We report here that stimulation of β-adrenergic receptors by (-)isoproterenol in rat cerebral cortical brain slices increases the density of serotonin$_2$ receptor binding sites. Modulation of serotonin receptors by norepinephrine could represent an important component of central serotonergic and noradrenergic nervous system interaction.

Methods and Results

Cerebral cortical slices (0.26 x 0.26 x 1-2 mm) were prepared from 12-15 male Sprague-Dawley rats (150-250g) as described previously (Dibner and Molinoff, 1979) and immediately transferred to 250 ml oxygen-saturated physiologic buffer (Kakiuchi and Rall, 1968) containing 0.1% (w/v) bovine serum albumin (Sigma Chemical Co.) at 37°C and preincubated for 10-50 minutes, during which the buffer was continuously gassed with 95% O_2 and 5% CO_2. Preincubated slices were centrifuged at 200g for 30 seconds, the supernatant aspirated, and the slices resuspended in 40 ml of fresh, gassed buffer at 37°C. Slices were equally aliquoted into control and test groups, and each group was diluted to a total volume of 150 ml with 37°C gassed buffer containing 0.5 mM sodium metabisulfite antioxidant (Sigma Chemical Co.) with or without 100 μM (-) isoproterenol (+) bitartrate (Sigma Chemical Co.). The incubation buffer was maintained at 37°C in a shaking incubator and gassed (95% O_2 / 5% CO_2) throughout each experiment. Following the incubation, the slices were diluted two-fold with ice-cold isotonic buffered saline and immediately homogenized using a Brinkman Polytron. Membranes for binding assays were then prepared according to Bylund and Snyder (1976) and protein concentrations were determined by the method of Lowry et al. (1951).

β-adrenergic and $5HT_2$ receptor binding to cerebral cortical membranes (430-600 μg protein) were determined using [^{3}H]-dihydroalprenolol ([^{3}H]-DHA, Amersham, 50.0 Ci/mmole) and [^{3}H]spiperone (Amersham, 17.0 Ci/mmole), respectively, as described elsewhere (Scott and Crews, 1983; Crews et al., 1983). Nonspecific

[^{3}H]-DHA binding was determined in the presence of 5.0 μm l-alprenolol-d-tartrate (Sigma Chemical Co.), and nonspecific [^{3}H]-spiperone binding was determined in the presence of 25 μM ketanserin tartrate (Janssen Pharmaceuticals).

Incubation of cerebral cortical slices with 100 μM (-)isoproterenol reduced the specific binding of the β-receptor antagonist [^{3}H]-DHA. We found a 26.5 ± 2.9% ($p<0.01$; $n = 6$) reduction after 30 minutes (Figure 14). The reduction in [^{3}H]-DHA binding was maintained throughout a 120 minute incubation. β-receptor binding in control slices was not significantly changed over this time course. In contrast, the specific $5HT^2$ receptor binding of [^{3}H]-spiperone was increased in the slices incubated with 100 μM (-)isoproterenol. There was a 15.2 ± 5.1% ($p<0.01$; $n = 6$) increase in $5HT_2$ receptor binding after 30 minutes of incubation (Figure 14). The isoproterenol-induced increase in HT_2 receptor binding was maintained for at least 120 minutes and control binding did not change over this time course.

To determine if these changes in specific binding were due to changes in the density of receptors, i.e. B_{max}, or the apparent affinities of these receptors for their respective ligands, i.e. K_d, the specific binding of several different concentrations of tritiated ligand was determined and saturation analyses performed as described elsewhere (Scott and Crews, 1983; Crews et al., 1983; Rosenthal, 1967; Scatchard, 1948). These studies indicated that the density of serotonergic$_2$ receptors was increased by 30.5 ± 7.3% ($p<0.001$; $n = 6$) in the slices incubated with (-)isoproterenol. In addition, the dissociation constant (K_2) for [^{3}H]-spiperone was increased by 53.5 ±

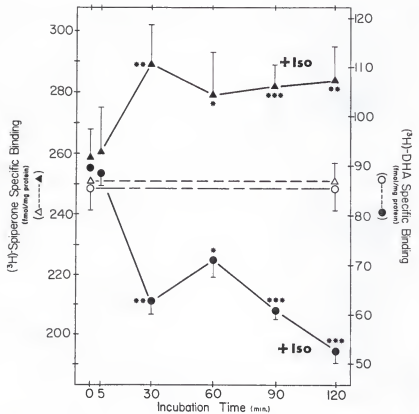

Figure 14. Time course of isoproterenol-induced changes of β- and $5HT_2$-receptor binding sites in rat cerebral cortical membranes. Slices were incubated for various times at 37°C with or without 100 μM (-)isoproterenol, after which membranes were prepared and assayed with a single concentration of [^{3}H]-labeled ligand. β-adrenergic receptor binding was assayed with 1.45 nM [^{3}H]dihydroalprenolol ([^{3}H]DHA). $Serotonin_2$ receptor binding was assayed with 1.91 nM [^{3}H]spiperone. Assays were terminated by filtration through Whatman GF/B filters, and protein concentrations of 430-470 μg/ml and 430-470 μg/2 ml were used for β- and $5HT_2$-receptor assays, respectively. Specific radioligand binding to control membranes did not vary significantly with incubation time, and the control values ± S.E.M. averaged over the entire incubation period were 85.4 ± 4.1 fmol/mg protein for [^{3}H]DHA and 251 ± 5 fmol/mg protein for [^{3}H]spiperone. Each point is the mean ± S.E.M. of four separate determinations for [^{3}H]DHA binding and six separate determinations for [^{3}H]spiperone binding, each determined in triplicate. The data shown are typical of results obtained in six experiments of similar design. Student's t-test was used to evaluate differences for significance. *P<0.05, **P<0.01, and ***P<0.001 when compared to controls.

19.8% ($p<0.05$; $n = 6$) (Figure 15; Table 5). Incubation with isoproterenol decreased the B_{max} for [^{3}H]-DHA by 19.6 ± 0.5% ($p<0.01$; $n = 6$). Isoproterenol treatment also decreased the apparent affinity for [^{3}H]-DHA although this could have been due to residual isoproterenol in the membranes.

To investigate the pharmacological specificity of the isoproterenol-induced changes in $5HT_2$ and β receptor binding, slices were coincubated with 100 μM (-)isoproterenol and 50 μM sotalol-HCl (Mead-Johnson), a specific β-receptor antagonist. The coincubation with sotalol prevented the isoproterenol-induced changes in β and $5HT_2$-receptor density (Table 2). This suggests that changes in both β and $5HT_2$ receptor B_{max} are mediated by β-receptor stimulation. The lowered binding affinity of [^{3}H]-DHA in sotalol-treated tissue could be due to residual drug in the membranes. The reason for the change in affinity for [^{3}H]-spiperone in the various groups is not clear.

In order to determine if β-receptor stimulation affects the binding of other receptor sites, we measured serotonin$_1$ ($5HT_1$) and α_2-adrenergic receptor binding after incubating cerebral cortical slices with 100 μM (-)isoproterenol. Serotonin$_2$ receptor binding was determined using membranes preincubated in 10 μM pargyline-HCl (Sigma Chemical Co.) for 25 minutes at 37°C. The membranes (740-800 μg protein) were incubated with 1.0 nM [^{3}H]-serotonin (Amersham, 12.3 Ci/mmole) and 10 μM pargyline in 1 ml 50 mM Tris-HCl, pH 7.7, for 10 minutes at 37°C. Total binding was determined in triplicate and nonspecific binding in duplicate in the presence of 20 μM serotonin-creatinine sulfate (Sigma Chemical Co.). α_2-adrenergic

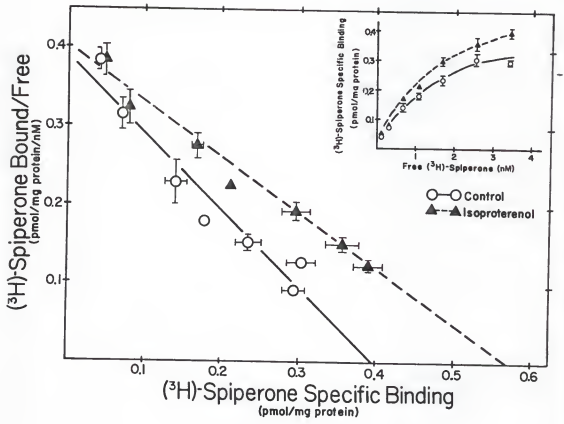

Figure 15. Saturation analysis of specific $5HT_2$-receptor binding to rat cerebral cortical membranes. Slices were incubated for 60 minutes at 37°C with or without 100 μM (-)isoproterenol. Membranes were prepared and six separate saturation analyses performed with five concentrations of [^{3}H]spiperone ranging from 0.54 to 4.0 nM. Assays were terminated by filtration through Whatman GF/C filters and a protein concentration of 300 μg/ml was used. Each point is the mean ± S.E.M. of six separate values, each determined in triplicate. Similar results were obtained in six other experiments of similar design. In each experiment, the B_{max} of [^{3}H]DHA binding in isoproterenol incubated slices was significantly decreased from that of control slices.

Table 5. Effects of sotalol on isoproterenol-induced changes in cerebral cortical $5HT_2$ and β receptor binding.

	[^{3}H]-Spiperone		[^{3}H]-Dihydroalprenolol	
Treatment	B_{max} (fmol/mg protein)	K_d (nM)	B_{max} (fmol/mg protein)	K_d (nM)
Control	233 ± 6	0.86 ± 0.09	122 ± 6	2.16 ± 0.15
ISO	304 ± 17***	1.32 ± 0.17*	98 ± 1**	4.88 ± 0.27***
ISO + Sotalol	223 ± 13	0.48 ± 0.07*	134 ± 10	6.59 ± 0.63***
Sotalol	244 ± 20	0.64 ± 0.06	134 ± 2	1.90 ± 0.12

*P<0.05, **P<0.01, ***P<0.001 when compared to control.

Cerebral cortical brain slices were incubated for 60 minutes in the presence of 100 μM (-)isoproterenol (ISO), 50 μM sotalol, or 100 μM (-)isoproterenol plus 50 μM sotalol. Control slices were incubated throughout the 60 minutes period with an equal volume of 0.5 mM sodium metabisulfite vehicle. Saturation analyses were performed on membrane homogenates of the slices using concentrations of [^{3}H]-Spiperone and [^{3}H]-DHA ranging from 0.5 to 4.0 nM and 0.40 to 10.0 nM, respectively. Values represent the mean ± S.E.M. of three or four separate Rosenthal determinations.

receptor binding was determined by incubating membranes (450 μg protein) with 2.96 nM [^{3}H]-clonidine (Amersham, 24.0 Ci/mmole) in 1 ml 50 mM Tris-HCl, pH 8.0, for 30 minutes at 25°C. Total binding was determined in triplicate and nonspecific binding in duplicate in the presence of 5.0 μM clonidine-HCl (Boerhinger Ingelheim). Both serotonin$_1$- and α_2-receptor binding assays were terminated by rapid filtration through Whatman GF/B filters with four washings with 5 ml ice-cold 50 mM Tris-HCl, pH 8.0. Radioactivity was counted as described previously. Serotonin$_1$ receptor binding was not significantly altered after incubating slices for 60 or 120 minutes with isoproterenol (data not shown). In agreement with previous reports (Maggi et al., 1980), α_2-receptor binding was increased 21.6 ± 2.3% ($p<0.001$; $n = 6$) in slices incubated for 90 minutes with isoproterenol.

Although serotonin$_2$ sites have previously been shown to be separate from α_2 adrenergic sites, we have determined the apparent binding affinities of a number of serotonergic and adrenergic compounds at these sites and have clearly established a difference between these receptor sites. Binding of [^{3}H]-spiperone or [^{3}H]-clonidine to cerebral cortical membranes was determined as previously described in the presence of 4 or 5 concentrations of displacing agent ranging from 0.01 to 50.0 μM. The potency of each agent in inhibiting radioligand binding was taken as the inverse of the apparent inhibitory constant, K_i, where $K_i = IC_{50}/(1 + [^3H\text{-ligand}]/K_d)$. The IC_{50} (median inhibitory concentration) was calculated by probit analysis, and the K_d of [^{3}H]-spiperone binding was determined by

saturation analysis to be 0.86 nM. The K_d of [^{3}H]-clonidine binding was 2.0 nM, as determined by U'Prichard et al. (1977). The potency series in inhibiting [^{3}H]-spiperone binding was: spiperone (0.88 ± 0.22) ≥ ketanserin (1.20 ± 0.58) > MJ-13754-1 (3.04 ± 1.12) >> rauwolszine (1513 ± 312) > iprindole (2155 ± 190) > yohimbine (2578 ± 114) > clonidine (10356 ± 3399); the potency series in inhibiting [^{3}H]-clonidine binding was: clonidine (0.179 ± 0.037) ≥ rauwolszine (240 ± 19) ≥ yohimbine (278 ± 12) > MJ-13754-1 (4915 ± 135) > iprindole (8072 ± 983) > spiperone (17396 ± 1130) > ketanserin (> 10^6). Apparent K_i values ± S.E.M. are in units of nM in parenthesis. Both (-)isoproterenol and sotalol had extremely low affinities for the $5HT_2$ receptor with $K_i > 10^6$ nM in inhibiting specific [^{3}H]-spiperone binding.

Discussion and Summary

Our observation that 30 minutes of incubation with isoproterenol induced a down-regulation of β-adrenergic receptor binding is consistent with previous reports (Dibner and Molinoff, 1979). This down-regulation may be due to the induction of a high affinity state of the β receptor to which the isoproterenol remains essentially irreversibly bound (Dibner and Molinoff, 1979). This decrease in β receptor binding by isoproterenol has been shown in other studies to be reversed when membranes are incubated with GTP (Dibner and Molinoff, 1979).

The increase in $5HT_2$ receptor density with β-adrenergic stimulation could be due to an alteration in presynaptic release or an interaction of these two receptor types on the same cell. Since

dorsal and median raphe lessions and other alterations in serotonin stimulation do not alter $5HT_2$ receptor binding (Blackshear et al., 1981), an interaction of these two receptors on the same cell seems more likely. A direct interaction of isoproterenol and sotalol with the $5HT_2$ receptor is unlikely, since both have extremely low affinities for this site. Therefore, it appears that β receptor stimulation modulates $5HT_2$ receptors. This modulation could occur through changes in membrane fluidity, cyclic AMP dependent protein kinases or other possible mechanisms.

Whatever the mechanism, an interaction between β-adrenergic and serotonin$_2$ receptors has several important implications. Recent studies indicating that administration of β-adrenergic agonists enhances serotonergic behavioral responses suggests that adrenergic agonists may act in part by enhancing the sensitivity to serotonin (Ortmann et al., 1981; Cowen et al., 1982). Moreover, studies have indicated important interactions between central noradrenergic and serotonergic nervous systems in the actions of antidepressants. Electroconvulsive shock treatment (ECS) decreases β-adrenergic receptor binding (Bergstrom and Kellar, 1979) and the β-receptor stimulation of adenylate cyclase (Vetulani et al., 1976), whereas it increases $5HT_2$ receptor binding (Kellar et al., 1981), and serotonin-induced behavioral responses (Costain et al., 1979). It is possible that these enhanced behavioral responses by ECS are due to supersensitive $5HT_2$ receptors, which may in turn be due to enhanced stimulation of β receptors by norepinephrine. Indeed, studies have shown that depletion of brain norepinephrine prevents the ECS-induced

enhancement of serotonin-mediated behavior (Green and Deakin, 1983) and inhibition of catecholamine synthesis with α-methyl-p-tyrosine prevents the ECS-induced increase in $5HT_2$ receptor number in the cerebral cortex (Green et al., 1983). In contrast to ECS, chronic tricyclic antidepressant treatment decreases $5HT_2$ receptor binding (Peroutka and Snyder, 1980). The role of serotonergic nerves in this down-regulation is ambiguous since depletion of brain serotonin does not prevent the reduction of $5HT_2$ receptor number by chronic amitriptyline (Clements-Jewery and Robson, 1982). It is possible that chronic antidepressant treatment acts through the noradrenergic systems to down-regulate $5HT_2$ receptors via a β-$5HT_2$ receptor interaction. In addition to antidepressant treatments, a β-$5HT_2$ receptor interaction has other important implications in the regulation of the cardiovascular system, sleep patterns, and pituitary hormone release.

In summary, we have shown that incubation of cerebral cortical slices with 100 μM (-)isoproterenol decreased the density of β adrenergic receptors and increased that of $5HT_2$ receptors. These changes appear to have been mediated by β receptor stimulation since they were prevented by the selective β receptor antagonist sotalol. In addition, isoproterenol increased α_2-adrenergic receptor binding, but had no significant effect on $5HT_1$ receptor binding in cerebral cortical slices.

CHAPTER FIVE
DOWN-REGULATION OF SEROTONIN$_2$, BUT NOT β-ADRENERGIC RECEPTORS DURING CHRONIC AMITRIPTYLINE TREATMENT IS INDEPENDENT OF SEROTONIN$_2$ AND β-ADRENERGIC RECEPTOR STIMULATION

Introduction

Antidepressants are thought to mediate their therapeutic effects through a progressive down-regulation of β-adrenergic (Banerjee et al., 1977; Bergstrom and Kellar, 1979), α_2-adrenergic (Crews and Smith, 1978; Svensson and Usdin, 1978) and serotonergic$_2$ (Peroutka and Snyder, 1980) receptors in the brain. The down-regulation of β receptors by antidepressants appears to be due to their enhanced stimulation by increased synaptic concentrations of norepinephrine (Wolfe et al., 1978). However, the mechanisms by which antidepressants down-regulate serotonin$_2$ ($5HT_2$) receptors is unknown. Recent studies have shown that lesions of central serotonergic nerve terminals do not prevent the decrease in cerebral cortical $5HT_2$ receptors by the antidepressant amitriptyline (Clements-Jewery and Robson, 1982). Thus, the amitriptyline-induced down-regulation of the $5HT_2$ receptor may be independent of changes in the availability of serotonin.

It is possible that the down-regulation of $5HT_2$ receptors by antidepressants is mediated through interactions with the adrenergic nervous system. There are several studies suggesting interactions

between central serotonergic and adrenergic transmission which may be important in antidepressant action. Lesions of central serotonergic axons by 5,7-dihydroxytryptamine prevent the down-regulation of β-adrenergic receptors by the antidepressant desipramine (Brunello et al., 1982). In addition, stimulation of β receptors in cerebral cortical slices increases $5HT_2$ receptor density (Scott and Crews, 1985). Furthermore, it has been shown that α_2-adrenergic antagonists accelerate and enhance the down-regulation of both β-adrenergic (Scott and Crews, 1983) and $5HT_2$ (Crews et al., 1983) receptors by antidepressants. These data indicate that the down-regulation of biogenic amine receptors by antidepressants may be related and interdependent.

In the present study, we investigated the role of serotonin in the regulation of central $5HT_2$ receptors. We found that coadministration of the $5HT_2$ receptor antagonist nefazadone with amitriptyline over a three week course did not prevent the down-regulation of $5HT_2$ receptors in the cerebral cortex. This suggests that the antidepressant-induced down-regulation of $5HT_2$ receptors is not mediated through enhanced serotonergic synaptic transmission. Furthermore, we found that depletion of central stores of norepinephrine and serotonin with reserpine increased β receptor binding in the cerebral cortex and hippocampus, but did not affect $5HT_2$ receptor binding. Thus, it appears that central $5HT_2$ receptor number is not regulated by changes in the availability of serotonin.

To determine if antidepressant-induced down-regulation of central $5HT_2$ receptors is mediated through a β-$5HT_2$ receptor interaction, we

coadministered amitriptyline in combination with propranolol. We found that coadministration of propranolol with amitriptyline for three weeks prevented the down-regulation of cerebral cortical β receptors, but did not alter the down-regulation of $5HT_2$ receptors. Thus, the $5HT_2$ receptor down-regulation by amitriptyline appears to be independent of the β-adrenergic receptor down-regulation.

Methods

Animal Treatment and Tissue Preparation

Male Sprague-Dawley rats (250-350 g) were housed in a well-ventilated room with food and water available ad libitum. Lights were on between 800 and 1600 hours. Drugs were administered by i.p. injection usually at about 9:00 a.m. Animals were sacrificed by decapitation 24 hours after the final drug injection, their brains rapidly removed and placed in ice-cold phosphate-buffered saline, pH 7.4. The cerebral cortices with frontal lobes and hippocampi were dissected and placed in 5 ml ice-cold 50 mM Tris(hydroxymethyl)-aminomethane (Tris) adjusted to pH 7.5 with HCl. Samples were then homogenized with a Brinkman Polytron at setting 50 for approximately 30 seconds. The homogenates were centrifuged at 49,000 g for 15 minutes at 4°C, the supernatant discarded, and the pellets rehomogenized in 5 ml fresh 50 mM Tris. After a second centrifugation at 49,000 g for 15 minutes, the samples were homogenized as before in 20 ml of 50 mM Tris/g wet wt. of tissue.

Determination of [^{3}H]dihydroalprenolol Binding to Homogenates

In this study, β-adrenergic receptor binding is defined as the specific binding of [^{3}H]dihydroalprenolol ([^{3}H]-DHA) calculated as the difference between total [^{3}H]DHA binding and nonspecific [^{3}H]DHA binding in the presence of 5 μM l-alprenolol as described previously (Scott and Crews, 1983). Approximately 12 hours elapsed between animal sacrifice and β-adrenergic receptor binding determinations, during which the tissue was stored at 4°C. Preliminary studies indicated that this storage was sufficient to prevent alterations in receptor binding. Binding of [^{3}H]-DHA was determined by incubating 150 μl of tissue homogenate with 850 μl Tris, pH 8.0 containing a single concentration of [^{3}H]-DHA (1.5-2.5 nM) for 20 minutes at 25°C. Previous studies have indicated that binding is at equilibrium under these conditions. Total binding was determined in triplicate and nonspecific binding in duplicate. Assays were terminated by rapid vacuum filtration through Whatman GF/C filters, and membranes were subsequently washed free of residual [^{3}H]-DHA x 4 with 5 ml ice-cold Tris, pH 8.0. The filters with bound membranes were then placed in vials containing 7 ml of Liquiscint scintillation fluid (National Diagnostics) and shaken for at least 1 hour. Radioactivity was determined with a Beckman LS 7500 scintillation counter with a counting efficiency of approximately 35%.

Determination of [^{3}H]-spiperone Binding to Homogenates

We define $5HT_2$ receptor binding as the specific binding of [^{3}H]-spiperone calculated as the difference between total binding and nonspecific binding in the presence of 25 μM ketanserin. Binding of

[^{3}H]-spiperone was determined by incubating 150 μl of tissue homogenate with 1850 μl of 50 mM Tris, pH 6.9 containing a single concentration of [^{3}H]-spiperone (2.0-2.5 nM) for 15 minutes at 37°C as described previously (Crews et al., 1983). Previous studies indicated that binding is at equilibrium under these conditions. Total binding was determined in triplicate and nonspecific binding was determined in duplicate. Assays were terminated, filters washed, and radioactivity determined as described above for the β receptor assay. No more than 6 hours elapsed between animal sacrifice and the assay of $5HT_2$ receptor binding.

Statistical Analysis

Data are expressed as the mean ± S.E.M. The Student's t-test was used to evaluate the differences between two means for significance. The criterion for significance was $P<0.05$. K_i values for nefazadone and amitriptyline were determined as described previously (Crews et al., 1983).

Drugs

The following drugs were kindly donated by the companies indicated: amitriptyline-HCl (Merck, Sharpe and Dohme Research Labs), nefazadone (Mead-Johnson Pharmaceutical Co.), and ketanserin tartrate (Janssen Pharmaceuticals). L-alprenolol-D-tartrate and propranolol-HCl were purchased from the Sigma Chemical Co. Reserpine was obtained as an injectible solution from CIBA Pharmaceutical Company.

The reserpine was provided in an aqueous solution containing 10% (v/v) dimethylacetamide, 10 mg/ml adipic acid, 0.1 mg/ml versene, 1.0% (v/v) benzyl alcohol, 5% (v/v) polyethylene glycol 300, 0.5 mg/ml ascorbic acid, and 0.1 mg/ml sodium sulfite. In the amitriptyline/propranolol experiments, both drugs were dissolved in 5 mg/ml in phosphate buffered saline with 10% (v/v) ethanol. In the amitriptyline/nefazadone experiments, both drugs were dissolved in deionized water (2.5 mg/ml for amitriptyline; 12 mg/ml for nefazadone) by probe sonication at 60 watts for approx 15 minutes. In each experiment, control animals received i.p. injections of vehicle which were identical in volume and composition to the solvents used in each test group with the exception of the reserpine experiment, where control animals received injections of physiologic buffered saline.

Results

Effects of Amitriptyline and Propranolol Administered Alone or in Combination on Cerebral Cortical β-Adrenergic and $5HT_2$ Receptor Binding

To determine the effect of β receptor blockade on the antidepressant-induced down-regulation of cerebral cortical β-adrenergic receptor binding, amitriptyline was administered alone or in combination with the β-receptor antagonist propranolol (Figure 16). Treatment with amitriptyline (10 mg/kg/day) alone for 3 weeks decreased β receptor binding by 8.9 ± 0.6% ($P<0.05$ when compared with controls). Coadministration of propranolol (10 mg/kg/day) with amitriptyline (10 mg/kg/day) for 3 weeks prevented the down-regulation of β receptors and induced an increase of 11.1 ± 3.5% ($P<0.01$) in β receptor binding. Administration of propranolol alone (10 mg/kg/day)

for 3 weeks increased β receptor binding in the cerebral cortex by 17.3 ± 5.1% ($P<0.01$).

In the above experiments, $5HT_2$receptor binding was also determined in the same cerebral cortical samples used for the β receptor binding (Figure 17). Amitriptyline (10 mg/kg/day) administered alone for the 3 week period decreased cerebral cortical $5HT_2$ receptor binding by 19.0 ± 2.1% ($P<0.01$ when compared to control binding). In contrast to the change in β receptor binding, coadministration of propranolol (10 mg/kg/day) with amitriptyline (10 mg/kg/day) did not prevent the down-regulation of $5HT_2$ receptors, and a 21.8 ± 4.5% decrease in $5HT_2$ receptor binding was observed ($P<0.01$). Propranolol (10 mg/kg/day) when administered alone for 3 weeks increased $5HT_2$ receptor binding from controls by 12.0 ± 5.6% ($P<0.05$).

Effects of Amitriptyline and Nefazadone Administered Alone or in Combination on Cerebral Cortical β-Adrenergic and $5HT_2$ Receptor Binding

In order to investigate the effects of serotonergic receptor blockade on amitriptyline induced changes in β-adrenergic and $5HT_2$ receptors, we administered amitriptyline (10 mg/kg/day) alone or in combination with nefazadone (50 mg/kg/day). Nefazadone has a high affinity for both $5HT_2$ receptors (IC_{50} 480 nM; Taylor et al., 1985) and $5HT_2$ receptors ([^{3}H]-spiperone K_i = 3.04 ± 1.12 nM). Furthermore, nefazadone has been shown to antagonize a variety of serotonergic responses (Hyslop et al., 1984; Taylor et al., 1985). Treatment with amitriptyline (10 mg/kg/day) alone for 3 weeks decreased β receptor binding by 7.0 ± 1.6% ($P<0.10$ when compared with controls, Figure 18). Administration of amitriptyline in combination with nefazadone reduced

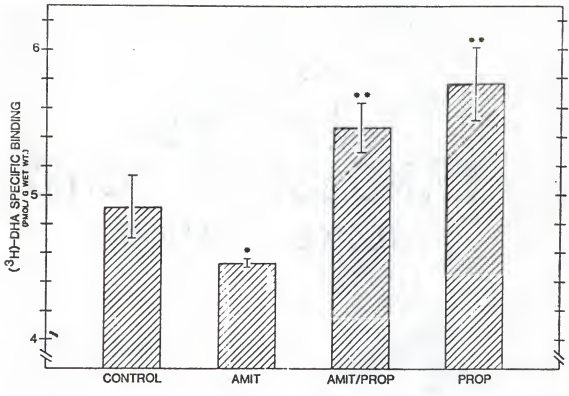

Figure 16. Effects of amitriptyline (Amit., 10 mg/kg/day) and propranolol (Prop., 10 mg/kg/day) administered alone or in combination for 21 days on cerebral cortical β receptor binding. Values are the mean ± SEM of specific [^{3}H]DHA binding to cerebral cortical membranes from drug-treated animals and vehicle-treated controls. The concentration of [^{3}H]DHA used was 2.3 nM. Nonspecific binding was determined in the presence of 5 μM l-alprenolol as described in Methods. Each group consisted of 6 animals. *P<0.05; **P<0.01 when compared to control values.

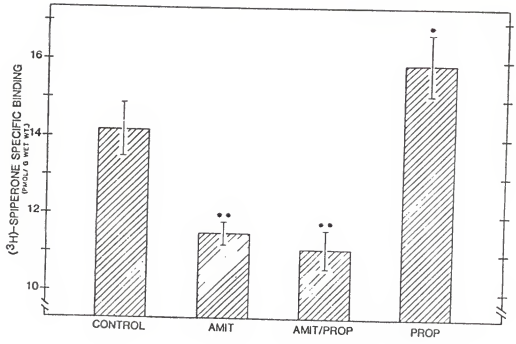

Figure 17. Effects of amitriptyline (Amit., 10 mg/kg/day) and propranolol (Prop., 10 mg/kg/day) administered alone or in combination for 21 days on cerebral cortical $5HT_2$ receptor binding. Values are the mean ± SEM of specific [^{3}H]spiperone binding to cerebral cortical membranes from drug-treated controls. The concentration of [^{3}H]spiperone used was 2.2 nM. Non-specific binding was determined in the presence of 25 μM ketanserin as described in Methods. Each group consisted of 6 animals. *P<0.05; **P<0.01 when compared to control values.

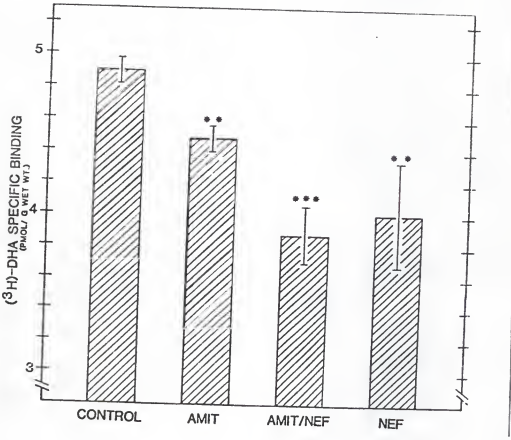

Figure 18. Effects of amitriptyline (Amit., 10 mg/kg/day) and nefazadone (Nef., 50 mg/kg/day) administered alone or in combination for 21 days on cerebral cortical β receptor binding. Values are the mean ± SEM of specific [^{3}H]DHA binding to cerebral cortical membranes from drug-treated animals and vehicle-treated controls, with 6 animals per group. Specific binding was determined using 1.3 nM [^{3}H]DHA as described in Methods. Nonspecific binding was determined in the presence of 5 μM l-alprenolol. **P<0.01; ***P<0.001 when compared to control values.

β receptor binding from controls by 21.0 ± 3.7% ($P<0.001$). In addition, administration of nefazadone alone decreased cerebralcortical β receptor binding by 18.3 ± 6.7% ($P<0.01$) when compared with controls.

Serotonin$_2$ receptor binding was also determined in cerebral cortical membranes (Figure 19). Amitriptyline (10 mg/kg/day) administered alone for 3 weeks decreased $5HT_2$ receptor binding by 20.5 ± 2.0% ($P<0.01$). Coadministration of nefazadone (50 mg/kg/day) with amitriptyline (10 mg/kg/day) decreased $5HT_2$ receptor binding by 22.6 ± 3.8% ($P<0.01$ compared to control values). In addition, administration of nefazadone (50 mg/kg/day) alone for 3 weeks decreased $5HT_2$ receptor binding by 25.6 ± 1.1% ($P<0.01$). Scatchard analysis indicated that this was due to a change in the maximum number of sites; i.e. control and nefazadone B_{max} values were 27.0 and 22.4 pmol/gm wet wt., respectively.

Effects of Reserpine on β-Adrenergic and $5HT_2$ Receptor Binding in the Cerebral Cortex and Hippocampus

To determine the effects of depletion of central biogenic amines on β and serotonin$_2$ receptor binding, rats were treated with reserpine (5 mg/kg/day, i.p.) for four days. This dose has previously been shown to deplete at least 90% of brain norepinephrine and serotonin after a single dose (Brodie et al., 1966). Reserpine treatment increased cerebral cortical β receptor binding by 26.0 ± 2.8% ($P<0.001$) and hippocampal β receptor binding by 46.6 ± 4.1% ($P<0.01$) when compared to respective controls (Figure 20, upper panel). In contrast, treatment with reserpine had no significant effect on 5HT

receptor binding in either the hippocampus or the cerebral cortex (Figure 20, lower panel).

Discussion

While many studies have focused on either adrenergic or serotonergic transmission in depression, it appears that interactions between these two nervous systems are important in the action of antidepressants. α_2-adrenergic receptor antagonists accelerate the antidepressant-induced down-regulation of both β-adrenergic and $5HT_2$ receptors in the brain (Scott and Crews, 1982; Crews et al., 1983). Lesions of serotonergic axons prevent the down-regulation of β-adrenergic receptors by desipramine (Brunello et al., 1982) and imipramine, but not by iprindol or mianserin (Gandolfi et al., 1984). Furthermore, lesions of serotonergic raphe nuclei increase β-adrenergic receptors in the cerebral cortex and hippocampus (Stockmeier et al., 1985), although serotonergic lesions appear to have little or no affect on $5HT_2$ receptor density (Barbaccia et al., 1983; Quik and Azimitia, 1983). In vitro studies in brain slices have indicated that stimulation of β-adrenergic receptors can increase $5HT_2$ receptor density (Scott and Crews, 1985). These studies suggest that there are interactions between β-adrenergic and serotonergic receptors and that the effects of antidepressants on these receptors may be related to these receptor interactions.

In order to investigate the role of serotonin stimulation of $5HT_2$ receptors on antidepressant-induced changes in cerebral cortical β-adrenergic and $5HT_2$ receptor down-regulation, we administered amitriptyline, a prototype antidepressant, with nefazadone, a much

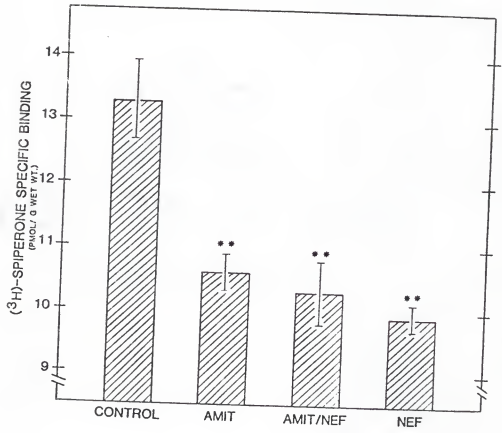

Figure 19. Effects of amitriptyline (Amit., 10 mg/kg/day) and nefazadone (Nef., 50 mg/kg/day) administered alone or in combination for 21 days on cerebral cortical $5HT_2$ receptor binding. Values are the mean ± SEM of specific [^{3}H] spiperone binding to cerebral cortical membranes from drug-treated animals and vehicle-treated controls, with 6 animals per group. Specific binding was determined using 2.3 nM [^{3}H]spiperone as described in Methods. Nonspecific binding was in the presence of 25 μM ketanserin. **P<0.01 when compared to control values.

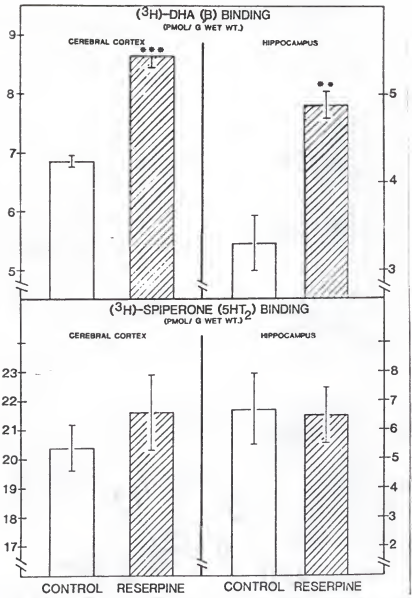

Figure 20. Effects of pretreatment with reserpine (5 mg/kg, i.p., per day for 4 days) on the binding of [^{3}H]-dihydroaprenolol ([^{3}H]DHA) (A) and [^{3}H]-spiperne (B). Clear columns represent controls; cross-hatched columns represent pretreatment.

more potent $5HT_2$ antagonist. Classically, receptor down-regulation is secondary to increases in agonist stimulation and antagonists causesupersensitivity and/or increases in receptor number (Creese and Sibley, 1981). Since amitriptyline is a potent inhibitor of serotonin uptake, it is possible that amitriptyline could enhance agonist activity at the $5HT_2$ receptor even though amitriptyline itself is an antagonist at this receptor. Amitriptyline, nefazadone and the combination of these two drugs down-regulated both $5HT_2$ and β-adrenergic receptors to a similar extent; i.e. the effects of the combination of the two drugs were not additive. This suggests that the down-regulation of the $5HT_2$ receptor is not dependent on stimulation by serotonin and is consistent with the finding that lesions of serotonergic neurons do not prevent the amitriptyline-induced down-regulation of $5HT_2$ receptors (Clements-Jewery and Robson, 1982). Several other antidepressants have been shown to decrease the density of $5HT_2$ receptors and many of these antidepressants are $5HT_2$ receptor antagonists. More over, serotonin antagonists which have no reported antidepressant activity, e.g. cyproheptadine, metergoline and methysergide, have been shown to down-regulate the $5HT_2$ receptor after a single dose (Blackshear et al., 1983). Amitriptyline and most other antidepressants require chronic treatment to decrease $5HT_2$ receptor binding. It is possible that the $5HT_2$ receptor behaves in a non-classical manner and is down-regulated by antagonists. Another possibility is that the $5HT_2$ receptor is part of a supramolecular structure which is regulated by serotonin acting at a separate site (Barbaccia et al., 1983). In any

case, the down-regulation of the $5HT_2$ receptor by amitriptyline does not appear to be dependent upon stimulation of the $5HT_2$ receptor by serotonin.

Our finding that coadministration of propranolol blocks the down-regulation of cortical β receptors by amitriptyline is consistent with the studies of Wolfe et al. (1978) who showed that desipramine induced down-regulation of cortical β-adrenergic receptors is prevented by coadministration of propranolol or lesions of central noradrenergic neurons with 6-hydroxydopamine. These studies suggest that the down-regulation of cortical β-adrenergic receptors during chronic antidepressant treatment is at least in part due to stimulation of cortical β receptors by norepinephrine. While coadministration of propranolol prevented the down-regulation of β-adrenergic receptors by amitriptyline, it did not alter the down-regulation of cerebral cortical $5HT_2$ receptors. Thus, it appears that the amitriptyline induced down-regulation of $5HT_2$ receptors is not mediated through the β-adrenergic system. Administration of propranolol alone increased both β and $5HT_2$ receptor binding. The up-regulation of β receptors is likely to be a supersensitive response to blockade and decreased stimulation. The mechanism of the $5HT_2$ receptor up-regulation is not clear, although changes in membrane fluidity by the lipophilic drug propranolol represent a possible explanation. Thus, the amitriptyline-induced decrease in cerebral cortical β receptor binding appears to require β receptor stimulation whereas the decrease in $5HT_2$ receptor binding appears to be independent of β receptor stimulation.

We found that depletion of central stores of norepinephrine and serotonin with reserpine increases β receptor binding, but does not alter $5HT_2$ receptor binding in the cerebral cortex or hippocampus (Figure 20). Moreover, others have demonstrated that electrolytic lesions of median and dorsal raphe nuclei do not alter central $5HT_2$ receptor binding (Blacksheer and Sanders-Bush, 1981). It is possible that $5HT_2$ receptors respond to changes in serotonin levels over a longer time course than heretofore studied or that these receptors receive serotonergic input from subpopulations of neurons not affected by reserpine or raphe lesions. Alternatively, it is possible that $5HT_2$ receptors are not innervated by serotonin, but respond to other endogenous transmitters or modulators. In fact, there is evidence that a peptide may represent the true endogenous ligand for $5HT_2$ recognition sites (Roth, Chuang and Costa, 1985). In any case, it is clear that the density of β-adrenergic receptors depends on the degree of stimulation by norepinephrine, whereas the $5HT_2$ receptor does not appear to adapt to alterations in serotonin. Thus, the effects of amitriptyline on β-adrenergic receptors appear to be related to changes in synaptic concentrations of norepinephrine whereas amitriptyline's actions on $5HT_2$ receptors appear to be independent of both serotonergic stimulation of $5HT_2$ receptors and noradrenergic β receptor stimulation.

CHAPTER SIX

RECIPROCAL CHANGES IN CEREBRAL CORTICAL α_1 AND β ADRENERGIC RECEPTOR RESPONSIVENESS AFTER CHRONIC TREATMENT WITH THE PROTOTYPE TRICYCLIC ANTIDEPRESSANT DESIPRAMINE: EVIDENCE FOR AN ALTERNATIVE HYPOTHESIS OF ANTIDEPRESSANT ACTION.

Introduction

Changes in central biogenic amine transmission have been implicated in the etiology and therapy of endogenous depression. The major current hypothesis of depression proposes that the therapeutic action of antidepressants is due to a progressive down-regulation in central β adrenergic receptor responsiveness (Sulser, 1979). All forms of antidepressant therapy, including tricyclic antidepressants, atypical antidepresants, monoamine oxidase inhibitors, and electroconvulsive shock have been shown to decrease the noradrenergic generation of cAMP in brain and, in most cases, the density of central β receptors, with a time course that parallels the onset of their clinical response; i.e. several weeks of administration are required (Sulser, 1979). Other psychotropic agents do not consistently show this effect. Furthermore, agents which deplete central stores of norepinephrine, e.g. reserpine, increase central β receptor density and responsiveness and can induce depressive symptoms in certain patients (Mendels and Frazer, 1974).

The decreased responsiveness of central β receptors by chronic antidepressant treatment appears to be due to enhanced adrenergic synaptic transmission. It has been shown that coadministration of the

β receptor antagonist propranolol with various antidepressants prevents the β receptor down-regulation (Wolfe et al., 1978; Scott and Crews, 1986). In addition, the down-regulation of central β receptors by the antidepressant desipramine (DMI) is prevented by prior lesions of adrenergic terminals with 6-hydroxydopamine (Wolfe et al., 1978). These findings suggest that enhanced adrenergic synaptic transmission by antidepressants decreases central β receptor responsiveness.

In addition to β receptors, α_1 receptors also appear to be important in adrenergic transmission and are present in greater density than β receptors in cerebral cortex. However, the effects of changes in adrenergic synaptic transmission on α_1 receptor responsiveness are not well defined. In contrast to β-adrenergic receptors, central α_1 receptors have received relatively minor attention in studies on antidepressant action since it has been found that chronic antidepressant treatment has no effect on central α_1 receptor density (Peroutka and Snyder, 1980). However, it is possible that α_1 receptor responsiveness may change with alteration in adrenergic synaptic transmission without corresponding changes in receptor density.

In the present study, we compared changes in cerebral cortical β and α_1 adrenergic receptor density and responsiveness during alterations in adrenergic synaptic transmission. β receptor responsiveness was determined by the noradrenergic generation of cAMP and α_1 receptor responsiveness by the noradrenergic stimulated phosphoinositide (PI) hydrolysis. Recent studies indicate that α_1 receptors are coupled to membrane PI hydrolysis in several brain

regions, including cerebral cortex, and this is a useful measure of α_1 receptor responsiveness (Gonzales and Crews, 1985). The prototype tricyclic antidepressant desipramine (DMI) was used to enhance, and reserpine was used to reduce, central adrenergic transmission. We found marked differences in changes in cerebral cortical β and α_1 receptor responsiveness during alterations in adrenergic synaptic transmission which changed the balance between these two receptor systems. It is possible that the etiology and treatment of endogenous depression are related to a change in the balance between β and α_1 receptor responsiveness in brain.

Methods

Male Sprague-Dawley rats (200-300g) were housed in a well ventilated room with food and water available ad libitum. Lights were on between 8:00 a.m. and 4:00 p.m. Drugs were administered either by i.p. injection or by continuous i.p. infusion via osmotic minipump (Alzet Corp., model 2ML2). Animals were sacrificed by decapitation and their brains rapidly removed and placed in ice-cold 50 mM Tris(hydroxymethyl)aminomethane, adjusted to pH 7.4 with HCl (Tris-HCl), prior to preparation of membranes for binding experiments or in warm 37°C physiologic buffer, pH 7.4 (Kakiuchi and Rall, 1968) prior to preparation of brain slices for stimulation experiments.

Determination of [^{3}H]-DHA and [^{3}H]-Prazosin binding to cerebral cortical membranes

After animal sacrifice and brain removal, cerebral cortices were dissected in thin slices to exclude underlying white matter. Frontal lobes were included in the dissection by making coronal cuts at the

level of the optic chiasm. The mean wet weight ± S.E.M. for this brain region was 523 ± 11 mg. The tissue was homogenized in 10 ml of ice-cold 50 mM Tris-HCl, pH 7.5 using a Brinkman polytron at setting 50 for approximately 30 seconds. The homogenates were centrifuged at 49,000xg for 15 minutes at 4°C, the supernatants were discarded, and the pellets were rehomogenized in 10 ml ice-cold fresh 50 mM Tris-HCl, pH 7.5. After a second centrifugation at 49,000xg for 15 minutes, the pellets were homogenized as before in 20 volume/g wet wt. of tissue. The membranes were stored on ice prior to receptor binding studies. Approximately 1 hour elapsed from animal sacrifice to assay of receptor binding.

β-adrenergic receptor binding to the cerebral cortical membranes was determined by a modification of the method of Bylund and Snyder (1976). The binding of [^{3}H]-dihydroalprenolol ([^{3}H]-DHA) was determined by incubating 150 μl of tissue homogenate with 850 μl of 50 mM Tris-HCl, pH 8.0 containing various concentrations of [^{3}H]-DHA for 20 minutes at 25°C. Total binding was determined in triplicate and nonspecific binding was determined in duplicate in the presence of 5 μM l-alprenolol. Specific binding was calculated as the difference between total and nonspecific binding. Saturation analyses were performed using concentrations of [^{3}H]-DHA ranging from 0.08 to 5.0 nM. After the samples were incubated, they were vacuum filtered over Whatman GF/C filters using a Brandel harvester and were washed four times with 5 ml of ice-cold 50 mM Tris-HCl, pH 8.0. The filters were placed in vials containing 8 ml of Liquiscint scintillation fluid (National Diagnostics) and shaken for at least 1 hour. Radioactivity

was determined with a Beckman LS 7000 scintillation counter with a counting efficiency of approximately 35%.

α_1-adrenergic receptor binding to cerebral cortical membranes was determined as the specific binding of [^{3}H]-prazosin. The binding of [^{3}H]-prazosin was determined by incubating 150 μl of tissue homogenate with 850 μl of 50 mM Tris-HCl, pH 7.4 containing various concentrations of [^{3}H]-prazosin for 30 minutes at 25°C. Total binding was determined in triplicate and nonspecific binding was determined in duplicate in the presence of 10 μM phentolamine. Specific binding was calculated as the difference between total and nonspecific binding. Saturation analyses were performed using concentrations of [^{3}H]-prazosin ranging from 0.04 to 25 nM. After the incubation, the samples were vacuum filtered through Whatman GF/C filters, washed, and radioactivity counted as described above.

Adrenergic stimulation of adenylate cyclase in cerebral cortical slices

Adenergic stimulation of adenylate cyclase in cerebral cortical slices was carried out by incubating the slices with norepinephrine or isoproterenol as modified by Vetulani et al. (1976). Cerebral cortices were dissected as described above. In addition, the dissected cortices were further cut into slices 0.35 mm thick in two perpendicular planes using a McIlwain tissue chopper. Each control or drug-treated group consisted of cerebral cortical slices obtained from 3 animals. Immediately following their preparation, the slices were transferred to flasks containing 50 ml physiologic buffer, pH 7.4 (Kakiuchi and Rall, 1968) at 37°C which had been bubbled with O_2/CO_2

(95:5). After the slices were gently dispersed, they were washed four times with fresh physiologic buffer at 37°C over a period of 10 minutes. The washed slices were then dispersed in 50 ml fresh physiologic buffer and incubated for 45 minutes at 37°C. The slices were continuously aggitated gently and bubbled with a slow stream of O_2/CO_2 during the incubation. After the 45 minutes incubation, the slices were allowed to settle and the supernatant buffer was removed and discarded. The slices were resuspended in 50 ml of fresh physiologic buffer containing 5 mM theophylline and were incubated an additional 15 minutes at 37°C with gentle aggitation and continuous bubbling of O_2/CO_2. Following the 15 minutes incubation, the slices were again allowed to settle and the supernatant buffer discarded. The slices were resuspended in 10 ml of fresh physiologic buffer containing 5 mM theophylline at 37°C. One ml aliquots of well-dispersed slices were then transferred to separate tubes containing 2 ml of physiologic buffer with 5 mM theophylline at 37°C for incubation with buffer alone for determination of basal cAMP production or with agonist. Incubation with buffer or agonist was carried out in triplicate. To begin the stimulation, 0.33 ml of physiologic buffer or of 10-fold concentrated agonist was added. Preliminary experiments indicated a dose-dependent increase in cAMP production stimulated by norepinephrine or isoproterenol, with maximum stimulation occurring with 100 μM of either agonist. Hence, it was decided to stimulate the slices with a final concentration of 100 μM of either agonist in all experiments. Immediately after addition of buffer or agonist, the tubes were rapidly gassed with a high pressure

stream of O_2/CO_2, tightly capped, and gently aggitated for 10 minutes at 37°C. Care was taken during this incubation to prevent the slices from settling on the bottom of the tubes. Time course experiments done previously indicated that maximum stimulation of cAMP production in cerebral cortical slices by either norepinephrine or isoproterenol occurred after 10 minutes of incubation. To stop the stimulation after the 10 minutes incubation, the slices were allowed to settle, and the supernatant buffer was aspirated and discarded. Preliminary experiments found no detectable cAMP in the supernatant buffer. Each tube containing the slices then received 3 ml boiling-hot 1 N HCl, and the slices were immediately homogenized with a Brinkman polytron at setting 50 for approximately 30 seconds. The homogenates were centrifuged at 27,000 x g for 30 min. The pellets and supernatants were separated, and the pellets were dissolved in 1 ml 0.5 N NaOH for protein determination (Lowry et al., 1951). Each incubation tube contained from 6.0 to 10.0 mg of protein. The supernatants were evaporated using a Savant rotary evaporator, and the residues remaining were dissolved in 1 ml 25 mM Tris-HCl, pH 7.0 containing 5 mM theophylline and saved for assay of cAMP.

The cAMP content of the dissolved residue samples was determined in triplicate using a protein binding assay modified from Brown et al. (1971). 50 μl of sample was incubated with 60 μl of 25 mM Tris-HCl, pH 7.0, 50 μl 0.8 nM [^{3}H]-cAMP (Amersham; 41 Ci/mmole), and 40 μl of cAMP-dependent protein kinase (1.25 mg/ml; Sigma Corp.; binding activity 0.09 pmol cAMP/mg) for 60 minutes in an ice-water bath. Preliminary experiments indicated that the binding of [^{3}H]-cAMP

reached equilibrium under these conditions. Total [^{3}H]-cAMP binding to the protein kinase was determined as above with the 50 μl of sample replaced with 50 μl of 25 mM Tris-HCl, pH 7.0. Nonspecific binding of [^{3}H]-cAMP was determined in the presence of 2.5 μM cAMP. To terminate the incubation after 60 minutes, 70 μl of hydroxyappetite (Sigma Corp.; diluted 1:1, v/v with 10 mM Tris-HCl, pH 7.0) was added to each sample, the sample vortexed and allowed to stand for at least 6 minutes. The samples were then vacuum filtered over Whatman GF/C filters and washed 3 times with 4 ml ice-cold 10 mM Tris-HCl, pH 7.0. The filters were placed flat in vials and shaken for at least 30 minutes in 1 ml 0.5 N HCl to dissolve the hydroxyappetite. Ten ml of Liquiscint scintillation fluid was added to each vial and shaken to form a homogeneous mixture. Radioactivity was determined as described above. Specific [^{3}H]-cAMP binding was calculated as the difference between total and nonspecific binding. The quantity of cAMP in each sample was determined by standard additions using the percent inhibition of specific [^{3}H]-cAMP binding by each sample. The standard curve was constructed by the same protocol as above using known concentrations of cAMP ranging from 2.5 nM to 500 nM. To confirm the specificity of this assay for cAMP, several of the unknown samples as well as samples with known cAMP content were incubated with 600 μg 3',5'-cAMP phosphodiesterase (Sigma Corp.; 0.02-0.05 U/mg) for 80 minutes at 37°C prior to assay. In all cases, preincubation with phosphodiesterase prevented the inhibition of [^{3}H]-cAMP binding to the protein kinase, confirming the specificity of this assay for cAMP.

Adrenergic stimulation of phosphoinositide hydrolysis in cerebral cortical slices

Adrenergic stimulation of phosphoinositide hydrolysis in cerebral cortical slices was performed according to the method of Gonzales and Crews (1985). Cerebral cortical slices were prepared as described above. Following the initial wash procedure, the slices were allowed to settle, excess buffer was aspirated, and the volume of the packed slices was estimated (usually 1.5 ml/cortex). The slices were transferred to a 50 ml conical tube and then diluted with 4 volumes of physiologic buffer to which had been added [^{3}H]-inositol (Amersham; 15.5 or 16.4 Ci/mmole) to give a final concentration of 0.1 to 0.3 μM. The tube was gassed with O_2/CO_2, tightly capped, and then incubated for 1 hour at 37°C with gentle aggitation. Care was taken to aggitate the slices enough during this incubation to prevent settling. To measure the amount of label incorporated into lipids, 0.2 ml of the slices was added to 0.8 ml ice-cold distilled water to stop the incorporation. The slices were then homogenized with a Tekmar SDT for 15 seconds at a medium setting. Aliquots taken for determination of protein by the method of Lowry et al. (1951) and for lipid extraction as described below.

Slices used for [^{3}H]-inositol phosphate release were allowed to settle and the medium was removed. The slices were washed twice with fresh physiologic buffer and then made up to a volume of 3 to 4 times the initial volume of packed slices. This volume is critical to maintain the protein concentration below 600 μg in a 50 μl aliquot. While gently aggitating the slices, 50 μl were transferred to Falcon 2069 polypropylene tubes (12 x 75 mm) containing 190 μl of physiologic

buffer with 10 mM LiCl substituted isotonically NaCl (8 mM final Li concentration). To start the reaction, 10 μl of agonist or buffer was added. The tubes were gassed with O_2/CO_2, capped tightly, and shaken in an incubator at 37°C for 60 minutes. The reaction was stopped by adding 1.0 ml of chloroform:methanol (1:2, v/v).

For determination of radioactivity incorporate into membrane lipids, the tubes containing the slices or homogenates received 1.0 ml chloroform:methanol (1:1, v/v). An additional 0.35 ml of distilled water and 0.35 ml of chloroform was added, and the tubes were capped tightly and shaken for 10 minutes. The tubes were then briefly centrifuged at low speed to separate the phases, and 0.75 ml of the upper aqueous phase was taken for Dowex chromatography as described below. The remaining upper phase and interphace were aspirated and discarded, and 200 μl of the chloroform layer was added to vials for determination of radioactivity incorporated onto membrane lipids. Five milliliters of OCS scintillation fluid (Amersham) was added to each vial, and radioactivity was determined as described above.

For analysis of [^{3}H]-inositol phosphate derived from the receptor-stimulated hydrolysis of phosphoinositides, the 0.75 ml samples of the aqueous phases were diluted to 3 ml with distilled water. One milliliter of a slurry of Dowex-1 (X8, 100 to 200 mesh; Sigma Corp.; 50% diluted, v/v) in the formate form was added to each diluted aqueous sample. The slurries containing the bound inositol phosphates were then poured into polypropylene columns with fritted disks. After allowing the liquid to drain, the resin was washed four times with 2.5 ml of 5 mM myoinositol to remove residual [^{3}H]-

inositol, and the eluate was discarded. Total inositol phosphates were eluted with five 1.0 ml washings with 0.1 M formic acid/1.0 M ammonium formate directly into vials. Each vial then received 10 ml of Liquiscint scintillation fluid and was shaken to form a homogenous mixture. Radioactivity was determined as described above. The release of [^{3}H]-inositol phosphates was calculated as the percent release relative to the [^{3}H]-phosphoinositide incorporated into the membrane lipid.

Drugs and reagents

The following drugs and reagents were used: l-[propyl-2,3-^{3}H]DHA (specific activity 33 Ci/mmole, radiochemical purity >98%; Amersham Corp., Arlington Heights, IL); [7-methoxy-^{3}H]prazosin (specific activity 60 Ci/mmole, radiochemical purity >98%; Amersham Corp.); [^{3}H]-inositol (specific activity 15.6 Ci/mmol, radiochemical purity >98%; Amersham Corp.); l-alprenolol-D-tartrate, prazosin hydrochloride, isoproterenol hydrochloride, norepinephrine-D-tartrate, reserpine hydrochloride, and DMI hydrochloride (Sigma Chemical Co., St. Louis, MO).

Drugs used in the in vitro simulation experiments, such as isoproterenol and norepinephrine, were dissolved in the physiologic buffer. Drugs used in binding experiments were dissolved in 50 mM Tris-HCl. DMI and reserpine were dissolved in propyleneglycol, ethanol, water (6:1:4,v/v). Reserpine required sonication at 50 watts for 5-10 minutes for dissolution. Reserpine was administered by i.p. injection, and DMI by continuous osmotic pump infusion (Alzet minipump model 2ML2). Animals designated as controls received vehicle

identical in volume and composition with the solvent injected with the dry in the corresponding test group.

Statistical analysis

Data are expressed as the mean ± S.E.M. The Student's t-test was used to evaluate the differences between two means for significance. The criterion for significance was $P < 0.05$.

Saturation analysis was determined by calculating best-fitting lines to the data points by least-squares linear regression analysis. Values for maximum binding, B_{max}, and apparent affinity, K_d, were determined using the Rosenthal derivation (Rosenthal, 1967) of Scatchard analysis (Scatchard, 1948). Standard curves for the cAMP assay and ED_{50} values for dose-response curves were determined using probit analysis as outlined by Goldstein (1964).

Results

Effects of acute and chronic desipramine administration on cerebral cortical α_1 and β adregenergic receptor binding

To determine the effects of acute and chronic treatment with desipramine (DMI) on cerebral cortical α_1 and β-adrenergic receptor binding, DMI (10 mg/kg/day) was administered for 1 and 14 days, respectively. Treatment with DMI for 1 day had no significant effect on either α_1 ([^{3}H]-prazosin) or β ([^{3}H]-DHA) receptor binding in the cerebral cortex (Figure 21, upper panel). Chronic treatment with DMI for 14 days also had no significant effect on cerebral cortical α_1 receptor binding (Figure 21, lower panel). Saturation analysis of cerebral cortical membranes from animals treated for 14 days indicated that chronic DMI treatment produced no change in the density (B_{max}) of

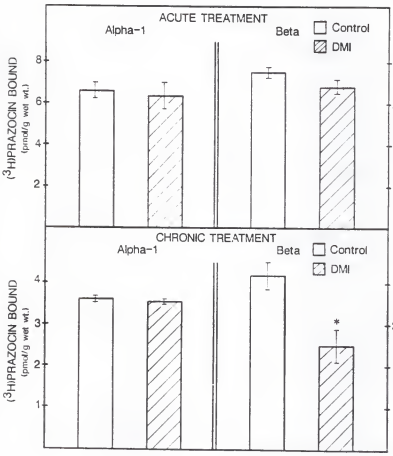

Figure 21. Effects of 1 day (upper panel) and 14 day (lower panel) administration of DMI (10 mg/kg/day) an cerebral cortical α_1 and β adrenergic receptor binding. Values are the mean ± S.E.M. of the specific binding of [^{3}H]-prazosin or [^{3}H]-DHA to cerebral cortical membranes prepared from vehicle or drug-treated animals for α_1 or β receptor binding, respectively. Membranes prepared from six animals in each group were incubated with a single concentration of [^{3}H]-prazosin (1.3-2.6 nM) or [^{3}H]-DHA (1.6-1.8 nM) for 30 or 20 minutes, respectively, at 25°C. Nonspecific [^{3}H]-prazosin binding was determined in the presence of 10 μM phentolamine; nonspecific [^{3}H]-DHA binding was determined in the presence of 5 μM l-alprenolol. *P<0.05 when compared with control values.

α_1 receptor binding sites (Table 6). In contrast, chronic DMI administration for 14 days significantly decreased cerebral cortical β-adrenergic receptor binding from 4.19 ± 0.36 pmol/g wet wt. to 2.72 ± 0.41 pmol/g wet wt. in control and DMI treated groups, respectively ($P < 0.05$; Figure 21, lowerpanel). Saturation analysis indicated that chronic treatment with DMI decreased the density of β receptor binding sites from 5.3 pmol/g wet wt. in control animals to 3.8 pmol/g wet wt. in DMI-treated animals (Table 6).

Effects of acute and chronic desipramine administration on cerebral cortical α_1 and β adrenergic receptor responsiveness

Incubation of cerebral cortical slices with norepinephrine increases plasma membrane phosphoinositide (PI) hydrolysis through an α_1 adrenergic receptor mediated pathway (Gonzales and Crews, 1985). In addition, incubation of cerebral cortical slices with norepinephrine or isoproterenol increases adenylate cyclase activity through β adrenergic receptor stimulation (Vetulani et al., 1976). Norepinephrine has greater efficacy than isoproterenol in stimulating adenylate cyclase due to an α receptor mediated facilitation of β receptor stimulated adenylate cyclase (Dumen et al., 1985).

In the present study, we determined the effects of acute and chronic DMI administration on cerebral cortical α_1 and β receptor responsiveness by measuring α_1 receptor stimulated PI hydrolysis and β receptor activation of adenylate cyclase in cerebral cortical slices prepared from animals treated with vehicle or DMI (10 mg/kg/day) for 1 and 14 days. Acute treatment with DMI for 1 day had no significant effect on β receptor stimulation of adenylate cyclase in cerebral

TABLE 6: Saturation analysis of [^{3}H]-prazocin (α_1) and [^{3}H]-DHA (β) binding to cerebral cortical membranes after chronic treatment with DMI

	Beta[b]		Alpha[c]	
	K_d(nM)	B_{max}($\frac{pmol}{gwet\ wt.}$)	K_d(nM)	B_{max}($\frac{pmol}{gwet\ wt}$)
CONTROL	0.63	15.1	0.47	5.3
DMI	0.73	14.3	0.74	3.8

[a]Animals were treated for 14 days with vehicle or DMI (10 mg/kg/day, i.p.).

[b]β receptor binding was determined as the specific binding of [^{3}H]-DHA at concentrations ranging from 0.08 nM to 5.0 nM.

[c]α receptor binding was determined as the specific binding of [^{3}H]-prazosin at concentrations ranging from 0.05 nM to 25 nM.

cortex (Table 7). Basal, isoproterenol-, and norepinephrine-stimulated cAMP production werenot significantly changed after acute DMI treatment. In addition, the difference between norepinephrine- and isoproterenol-stimulated cAMP production was esentially the same in both control and drug treated groups, indicating no change in the degree of α receptor facilitation of the β receptor activation of adenylate cyclase.

In contrast to acute treatment, chronic treatment with DMI (10 mg/kg/day) for 14 days significantly decreased adrenergic activation of adenylate cyclase in the cerebral cortical slices (Figure 22). Basal adenylate cyclase activity in the slices was unchanged after chronic DMI treatment. However, significant decreases in both isoproterenol- and norepinephrine-stimulated cAMP production were observed with chronic administration of DMI. In addition, no significant difference was observed between norepinephrine- and isoproterenol-stimulated cAMP production after chronic DMI administration, suggesting loss of the α receptor mediated facilitory input.

Similar to the effects on β receptor responsiveness, acute administration of DMI (10 mg/kg/day) for 1 day had no significant effect on cerebral cortical α_1 receptor responsiveness. Maximum norepinephrine-stimulated PI hydrolysis in cerebral cortex was 24.10 ± 1.04% [^{3}H]-IP release from control animals and 24.05 ± 0.67% [^{3}H]-IP release from animals acutely treated with DMI. The ED_{50} of cerebral cortical norepinephrine-stimulated PI hydrolysis was also unchanged after acute DMI treatment. In contrast to the effects on β receptor

TABLE 7: Effects of acute treatment with DMI on the noradrenergic generation of cAMP in cerebral cortical slices[a]

	BASAL (pmol/mg protein)	Isoproterenol (pmol/mg protein)	Norepinephrine (pmol/mg protein)	NE - ISO[b] (pmol/mg protein)
CONTROL	2.75 ± 0.44	13.21 ± 2.67	21.54 ± 1.68	8.33
DMI	2.96 ± 0.48	12.19 ± 1.30	21.12 ± 2.97	8.94

[a]Animals were treated with 1 dose of vehicle or DMI (10 mg/kg/day, i.p.), then sacrificed 24 hours later and basal, isoproterenol (ISO), and norepinephrine (NE) stimulated cAMP production in cerebral cortical slices determined as described in Methods. Slices were incubated with buffer alone for basal cAMP production, or with 100 μM of isoproterenol or norepinephrine. Values represent mean ± S.E.M. of 6 to 9 separate determination.

[b]The differences between norepinephrine and isoproterenol-stimulated cAMP production are thought to represent α-adrenergic receptor facilitation of β receptor stimulated adenylate cyclase activity (see text).

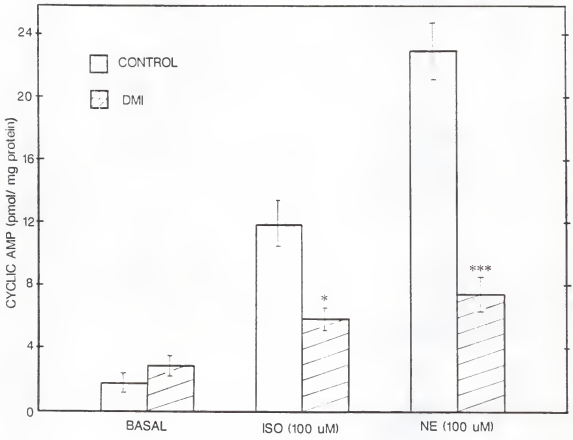

Figure 22. Effect of 14 day administration of DMI (10 mg/kg/day) on basal, isoproterenol (100 μM), and norepinephrine (100 μM) stimulated cAMP production in cerebral cortical slices prepared from vehicle and drug treated animals. Values are the mean ± S.E.M. of three to six separate determinations. Similar results were found in three independent experiments. *P<0.05; **P<0.01; ***P<0.001 when compared with controls.

responsiveness, chronic administration of DMI (10 mg/kg/day) for 14 days significantly increased cerebral cortical α_1 receptor responsiveness (Figure 23). Chronic DMI administration increased the maximum norepinephrine stimulated PI hydrolysis in cerebral cortical slices from a control value of 22.6 ± 0.1% [^{3}H]-PI release to 28.2 ± 0.7% [^{3}H]-PI release ($P<0.001$). In order to determine if chronic DMI administration affects the rate of α_1 receptor mediated PI hydrolysis in cerebral cortex, kinetic studies were carried out. Time course studies indicated that noradrenergic stimulated PI hydrolysis was more rapid in cerebral cortical slices from animals treated for 14 days with DMI as compared with controls (data not shown). Preliminary experiments indicated that neither the acute nor chronic DMI treatments affected the uptake of [^{3}H]-inositol by the cerebral cortical slices.

Effects of reserpine on cerebral cortical α_1 and β receptor binding and responsiveness

In order to determine the effects of diminished noradrenergic synaptic transmission on cerebral cortical α_1 and β receptor sensitivity, reserpine (5 mg/kg/day) was administered for 4 days. Previous studies have shown that this treatment depletes at least 90% of cerebral cortical stores of norepinephrine (Brodie et al., 1966). Reserpine administration significantly increased the densities of both α_1 and β adrenergic receptors in cerebral cortical membranes (Table 8). Similarly, reserpine increased cerebral cortical β receptor responsiveness (Figure 24). Reserpine treatment had no significant effect on basal cAMP production in cerebral cortical slices, but

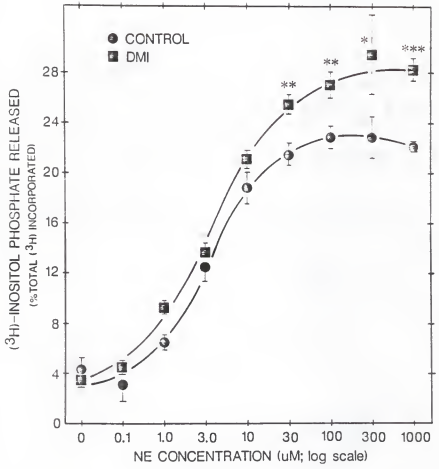

Figure 23. Effect of 14 day administration of DMI (10 mg/kg/day) on α_1 receptor stimulated [^{3}H]-inositol phosphate release in cerebral cortical slices prepared from vehicle and drug treated animals. Slices were preincubated with [^{3}H]-inositol for 60 minutes, washed, then stimulated with various concentrations of norepinephrine for 60 minutes. Values are the mean ± S.E.M. of three to six separate determinations. Similar results were found in three independent experiments. *P<0.05; **P<0.01; ***P<0.001 when compared with controls.

TABLE 8: Saturation analysis of [^{3}H]-prazosin (α_1) and [^{3}H]-DHA (β) binding to cerebral cortical membranes after acute reserpine treatment[a]

	Alpha$_1$[b]		Beta[c]	
	K_d(nM)	B_{max}($\frac{pmol}{gwet\ wt.}$)	K_d(nM)	B_{max}($\frac{pmol}{gwet\ wt}$)
CONTROL	0.61	12.7	0.37	4.64
RESERPINE	0.46	15.0	0.49	7.28

[a]Animals were treated for 4 days with vehicle or reserpine (5 mg/kg/day) by i.p. injection.

[b]α receptor binding was determined as the specific binding of [^{3}H]-prazosin at concentrations ranging from 0.05 nM to 25 nM.

[c]β receptor binding was determined as the specific binding of [^{3}H]-DHA at concentrations ranging from 0.80 nM to 10 nM.

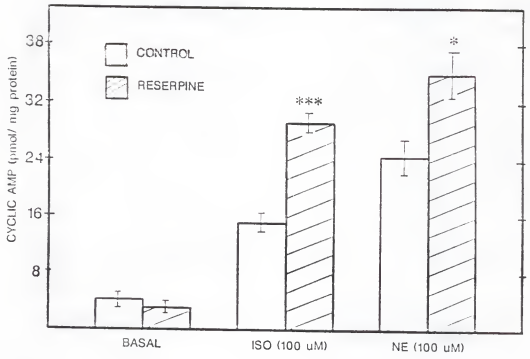

Figure 24. Effects of pretreatment with reserpine (5 mg/kg/day, i.p., for 4 days) on basal, isoproterenol (100 μM), and norepinephrine (100 μM) stimulated cAMP production in cerebral cortical slices prepared from vehicle and reserpine treated animals. Values are the mean ± S.E.M. of three separate determinations. *P<0.05; ***P<0.001 when compared with controls.

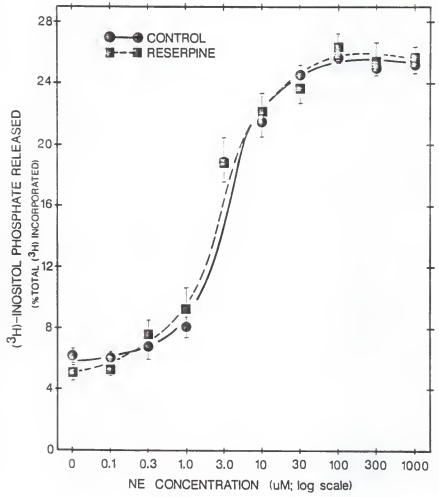

Figure 25. Effects of pretreatment with reserpine (5 mg/kg/day, i.p., for 4 days) on α_1 receptor stimulated [^{3}H]-inositol phosphate release in cerebral cortical slices from vehicle and reserpine treated animals. Slices were incubated with [^{3}H]-inositol for 60 minutes, washed, then stimulated with various concentrations of norepinephrine for 60 minutes. Values are the mean ± S.E.M. of six separate determinations.

increased isoproterenol-stimulated cAMP production from 15.14 ± 1.30 pmol/mg protein to 29.49 ± 1.42 pmol/mg protein ($P<0.001$) and increased norepinephrine-stimulated cAMP production from 24.60 ± 2.53 pmol/mg protein to 35.90 ± 3.43 pmol/mg protein ($P<0.05$). In contrast reserpine had no significant effect on cerebral cortical α_1 receptor responsiveness (Figure 25). Maximum norepinephrine stimulated PI hydrolysis in cerebral cortex was 22.05 ± 1.12 % [^{3}H]-IP release for controls and 21.51 ± 1.42% [^{3}H]-IP release for reserpine treated groups.

To determine the responses of α_1 and β adrenergic receptor system to diminished noradrenergic synaptic transmission over a longer time course, reserpine (2.5 mg/kg/day) was administered for 14 days. Administration of reserpine at this lower dose has previously been shown to deplete at least 90% of cerebral cortical stores of norepinephrine after a single treatment (Brodie et al., 1966).

Similar to the effects of acute reserpine treatment, chronic administration of reserpine significantly increased both α_1 and β adrenergic receptor density in membranes prepared from cerebral cortex (Table 9). The increases in density of both adrenergic receptor binding sites were greater after chronic treatment with reserpine than after acute administration. In addition, chronic treatment with reserpine also increased cerebral cortical β adrenergic receptor responsiveness (Figure 26). Treatment with reserpine for 14 days had no significant effect on basal cAMP production in slices prepared from cerebral cortex, but increased isoproterenol-stimulated cAMP production from 18.57 ± 1.86 pmol/mg protein to 53.33 ± 4.11 pmol/mg

TABLE 9: Saturation analysis of [^{3}H]-prazosin (α_1) and [^{3}H]-DHA (β) binding to cerebral cortical membranes after chronic reserpine treatment[a]

	Alpha$_1$[b]		Beta[c]	
	K_d(nM)	B_{max}($\frac{pmol}{gwet\ wt.}$)	K_d(nM)	B_{max}($\frac{pmol}{gwet\ wt.}$)
CONTROL	0.27	7.23	0.41	5.23
RESERPINE	0.19	9.40	0.61	9.94

[a]Animals were treated for 4 days with vehicle or reserpine (5 mg/kg/day) by i.p. injection.

[b]α receptor binding was determined as the specific binding of [^{3}H]-prazosin at concentrations ranging from 0.05 nM to 25 nM.

[c]β receptor binding was determined as the specific binding of [^{3}H]-DHA at concentrations ranging from 0.80 nM to 10 nM.

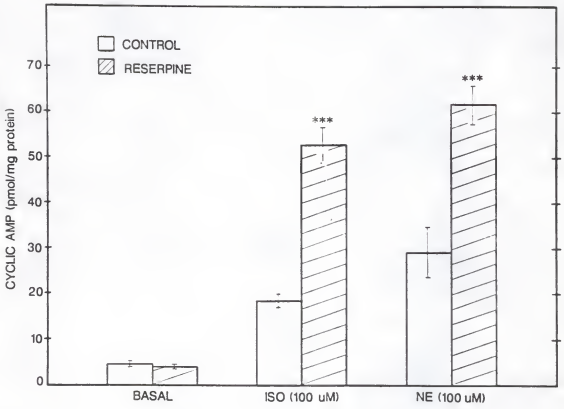

Figure 26. Effects of pretreatment with reserpine (2.5 mg/kg/day, i.p., for 14 days) on basal, isoproterenol (100 μM), and norepinephrine (100 μM) stimulated cAMP production in cerebral cortical slices prepared from vehicle and reserpine treated animals. Values are the mean ± S.E.M. of three separate determinations. *P<0.05; **P<0.01; ***P<0.001 when compared with controls.

protein ($P<0.05$) and increased norepinephrine-stimulated cAMP production from 29.21 ± 6.21 pmol/mg protein to 61.8 ± 4.70 pmol/mg protein ($P<0.05$). As with acute reserpine treatment, chronic administration of reserpine for 14 days had no significant effect on cerebral cortical α_1 receptor responsiveness (Figure 27). Maximum norepinephrine-stimulated PI hydrolysis in cerebral cortical slices was 25.18 ± 0.61% [^{3}H]-IP release for controls and 25.59 ± 0.82 % [^{3}H]-IP release for reserpine-treated groups.

Discussion

The findings of the present study indicate that cerebral cortical α_1 and β adrenergic receptors and their secondary messenger systems respond differently to alterations in adrenergic neurotransmission. Enhanced adrenergic synaptic transmission by chronic administration of the prototype tricyclic antidepressant DMI decreased β receptor density (Figure 21; Table 6) and responsiveness as determined by the noradrenergic generation of cAMP (Figure 22). In addition, the difference between norepinephrine- and isoproterenol-stimulated cAMP production was also reduced following chronic DMI treatment (Figure 22). Previous studies have suggested that the greater efficacy of the norepinephrine response is due to an α receptor mediated facilitation of β receptor stimulated adenylate cyclase activity (Dumen et al., 1985). Thus, our findings indicate that chronic treatment with DMI decreases both direct β receptor stimulated cAMP production and the facilitory input of α receptors in cerebral cortex. Acute administration of DMI for one day had no significant effect on β

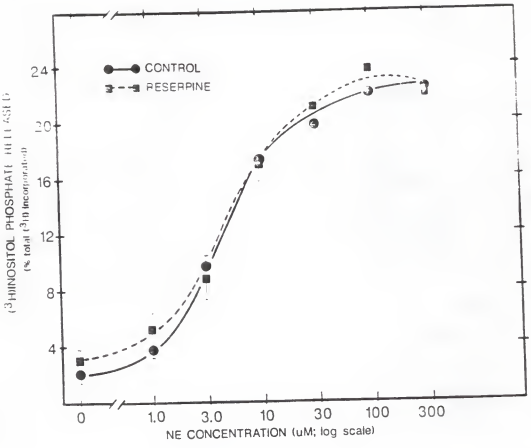

Figure 27. Effects of pretreatment with reserpine (2.5 mg/kg/day, i.p., for 14 days) on α_1 receptor stimulated [^{3}H]-inositol phosphate release in cerebral cortical slices from vehicle and resepine treated animals. Slices were preincubated with [^{3}H]-inositol for 60 minutes, washed, then stimulated with various concentrations of norepinephrine for 60 minutes. Values are the mean ± S.E.M. of three separate determinations.

adrenergic receptor density or on either component of the noradrenergic generation of cAMP in cerebral cortex (Figure 21).

In contrast to its effects on the β receptor system, chronic administration of DMI had no effect on cerebral cortical α_1 adrenergic receptor density (Figure 21; Table 6). However, chronic treatment with DMI significantly enhanced the efficacy of α_1 receptor stimulated PI hydrolysis in cerebral cortex (Figure 23). As with the β receptor system, acute administration of DMI for one day had no significant effect on the α_1 receptor system (Figure 21). This is most likely because the synaptic release of norepinephirne does not appear to be significantly altered with acute DMI administration (Crews and Smith, 1978).

The DMI-induced down-regulation of cerebral cortical β receptor density and responsiveness appears to be due to enhanced stimulation of β receptors by norepinephrine. Coadministration of propranolol with several chemically different antidepressants, including DMI, prevents the β receptor down-regulation (Wolfe et al., 1978; Scott and Crews, 1986), and lesions of central adrenergic terminals with 6-hydroxydopamine prevent the antidepressant-induced down-regulation of β receptors (Wolfe et al., 1978). In addition, coadministration of selective α_2 adrenergic receptor antagonists with antidepressants to augment synaptic release of norepinephrine accelerates and enhances β receptor down-regulation (Crews et al., 1981; Scott and Crews, 1983). Taken together, these findings suggest that the down-regulation of central β receptors by DMI and other antidepressants is due to enhanced synaptic release of norepinephrine.

In contrast, the mechanism of the DMI-induced increase in cerebral cortical α_1 receptor responsiveness is not clear. It is not likely that this up-regulation was due to chronic receptor blockade by DMI since depletion of norepinephrine with reserpine did not increase α_1 receptor responsiveness (Figures 25 and 27). It is also not likely that our observation was secondary to blockade of norepinephrine uptake in the brain slice preparations by residual DMI, as this would have increased the potency and not the efficacy of the response. Moreover, addition of DMI (1 μM) directly to cerebral cortical slices in vitro does not significantly affect norepinephrine-stimulated PI hydrolysis (Gonzales and Crews, 1985). Enhanced α_1 receptor stimulated PI hydrolysis was not an artifact due to increased [^{3}H]-inositol uptake by the brain slices in the assay, as preliminary experiments detected no difference in [^{3}H]-inositol uptake between control slices and those obtained from animals chronically treated with DMI. Kinetic studies indicated that the rate of cerebral cortical α_1 stimulated PI hydrolysis was increased after chronic DMI treatment, and this may, at least in part, account for the enhanced efficacy of the response. However, the mechanism of this effect is not yet clear.

In addition to investigating the effects of increased adrenergic synaptic transmission on cerebral cortical β and α_1 receptor systems, we also studied the effects of decreased adrenergic synaptic transmission on the density and responsiveness of these two receptor types. Administration of reserpine for 4 days to deplete central stores of norepinephrine increased both the density of cerebral

cortical β adrenergic receptors (Table 8) and the noradrenergic generation of cAMP (Figure 24). Similarly, cerebral cortical α_1 receptor density was increased after administration of reserpine for 4 days (Table 8). However, treatment with reserpine had no significant effect α_1 receptor responsiveness in cerebral cortex (Figure 25). Because of the possibility that α_1 receptor responsiveness adapts more slowly to decreased availability of norepinephrine, reserpine was administered over a longer period of 14 days. Reserpine was given at a lower dose for 14 days (2.5 mg/kg/day) than in the 4 day experiments (5 mg/kg/day) due to better animal tolerance. Previous experiments indicated that even the lower dose of reserpine was sufficient to deplete greater than 90% of total norepinephrine in brain (Brodie et al., 1966). Similar to acute administration, chronic treatment with reserpine for 14 days increased both β adrenergic receptor density (Table 9) and the noradrenergic generation of cAMP in cerebral cortex (Figure 26). While chronic reserpine administration also increased cerebral cortical α_1 receptor density (Table 9), it did not significantly alter α_1 receptor-mediated PI hydrolysis (Figure 27). In contrast to our findings, lesions of central adrenergic terminals with 6-hydroxydopamine have been shown to enhance α_1 receptor stimulated PI hydrolysis 14 days following the lesions (Kendall et al., 1985). The findings of the present study indicate that, unlike β receptor responsiveness, cerebral cortical α_1 receptor responsiveness is not affected by decreased availability of norepinephrine. Alternatively, α_1 receptor responsiveness may be regulated by cotransmitters which are affected by nerve terminal lesions, but not

by more selective depletion of norepinephrine with reserpine. It is possible that cerebral cortical α_1 receptors are innervated by adrenergic terminals which are not affected by reserpine but are lesioned by 6-hydroxydopamine. However, this is not likely since reserpine increased α_1 receptor density (Tables 8 and 9).

Both β and α_1 receptors appear to be important in central adrenergic synaptic transmission. These receptors may be present on different cells in the brain and transduce signals in separate adrenergic pathways. In addition, these two adrenergic receptors mediate opposite physiologic responses. β adrenergic receptors appear to be inhibitory for neuronal firing (DeMontigny et al., 1980), whereas α_1 adrenergic receptors appear to be excitatory (Menkes and Aghajanian, 1981). Thus, the balance between these two adrenergic receptors may be important in determining the net response to adrenergic transmission in brain. Our findings indicate that these two receptors respond differently to changes in the availability of norepinephrine and that changes in adrenergic synaptic transmisison alter the balance between these two receptor types. Enhanced adrenergic transnmission secondary to chronic administration of DMI shifts the α_1/β receptor balance in favor of the excitatory α_1 receptor. Diminished adrenergic transmission, secondary to depletion of norepinephrine by reserpine, shifts the α_1/β receptor balance in favor of the inhibitory β receptor (Figure 28).

The etiology and therapy of endogenous depression appears to be related to changes in central adrenergic transmission. The major hypothesis of depression to evolve over the past several years focuses

on β adrenergic receptor sensitivity. According to the β receptor hypothesis, depression arises from an abnormally supersensitive β adrenergic receptor system in brain and the clinical response to antidepressants is due to β receptor down-regulation (Sulser, 1979). It is possible, however, that it is a disruption in the balance between central β and α_1 receptor responsiveness that underlies the etiology of depression. Reserpine, which can induce depressive symptoms in certain patients, shifts the α_1/β receptor balance in favor of the β receptor, whereas the antidepressant DMI shifts this balance in favor of the α_1 receptor (Figure 28). Thus, it is possible that endogenous depression arises from excessive inhibitory β receptor tone relative to excitatory α_1 receptor tone and that antidepressants act by restoring the α_1/β receptor balance to normal.

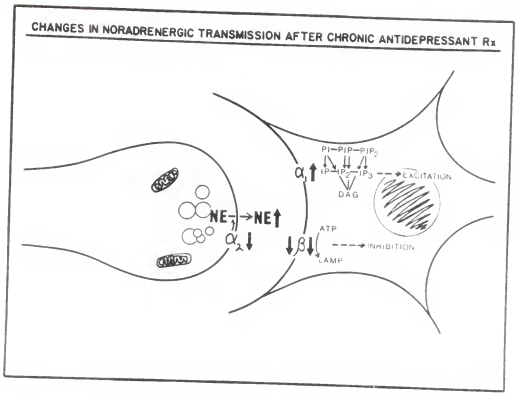

Figure 28. Hypothetical model for the $\alpha 1$ and β adrenergic receptor balance in a central adrenergic synapse. Norepinephrine (NE) released from the presynaptic terminal diffuses across the synapse to stimulate postsynaptic $\alpha 1$ and β adrenergic receptors. $\alpha 1$ receptor stimulation activates membrane phosphoinositide (PI) hydrolysis to release the secondary messengers inositol phosphates and diacylglyserol. Stimulation of the $\alpha 1$ receptor results in an excitation of postsynaptic neuronal firing. β receptor stimulation activates adenylate cyclase to produce the secondary messenger cAMP. Stimulation of the β receptor results in an inhibition of postsynaptic neuronal firing. Thus, the net effect of adrenergic synaptic transmission depends on the balance of $\alpha 1$ and β receptor responsiveness. Treatment with reserpine increases β receptor density and responsiveness and does not alter α_1 receptor responsiveness, shifting the balance in favor of the inhibitory β receptor. Chronic treatment with DMI elevates synaptic levels of norepinephrine which, in turn, decreases β receptor density and the β receptor stimulation of adenylate cyclase. By an unknown mechanism, chronic DMI treatment increases the $\alpha 1$ receptor stimulation of PI hydrolysis without changing $\alpha 1$ receptor density. Thus, chronic DMI administration shifts the balance between $\alpha 1$ and β receptor responsiveness from the inhibitory β receptor to the excitatory $\alpha 1$ receptor.

CHAPTER SEVEN
CONCLUSIONS AND SIGNIFICANCE

The etiology of endogenous depression and the mechanism of action of antidepressants are currently unknown. However, there is compelling evidence implicating central adrenergic and serotonergic nervous systems in the pathogenesis and therapy of this disease. Theories of depression have focused on α_2-adrenergic, β-adrenergic, and serotonergic $5HT_2$ receptors in brain. This dissertation presents the findings of experiments which further characterize the effects of antidepressants on central adrenergic and serotonergic receptor systems in order to further understand the mechanism or mechanisms of action of these drugs and the underlying pathology leading to endogenous depression. We have focused not only on these receptors individually, but have also explored interactions among these receptor systems as they relate to antidepressant action. We have found interactions between α_2 and β adrenergic receptors, α_2 and serotonergic $5HT_2$ receptors, α_1 and β adrenergic receptors, as well as β and $5HT_2$ receptors which may be important in the mechanism of action of antidepressants. Our findings presented in this dissertation have lead to an alternative hypothesis for the etiology and treatment of endogenous depression which focuses on the balance between α_1 and β adrenergic receptors in brain.

The major current β receptor hypothesis of depression postulates that the therapeutic action of antidepressants is mediated through a progressive down-regulation of central β-adrenergic receptors (Sulser, 1979). All forms of antidepressant therapy, including tricyclic and tetracyclic antidepressants, atypical antidepressants, monoamine oxidase inhibitors, and electroconvulsive shock (ECT) have been shown to decrease both the density of β receptors (Banerjee et al., 1977; Bergstrom and Kellar, 1979; Scott and Crews, 1983) and the noradrenergic generation of cAMP in brain (Vetulani et al., 1976) with a time course that parallels the onset of their therapeutic action; i.e. several weeks of treatment are required.

We have shown that the down-regulation of central β-adrenergic receptors by the tricyclic antidepressant amitriptyline is due to enhanced stimulation by norepinephrine. Coadministration of the β receptor antagonist propranolol with amitriptyline prevented β-receptor down-regulation by this antidepressant (Chapter Four). In addition, others have shown that coadministration of propranolol or lesions of central adrenergic terminals with 6-hydroxydopamine prevent central β receptor down-regulation by the tricyclic antidepressant desipramine (Wolfe et al., 1978). Taken together, these findings indicate that the down-regulation of central β receptors by antidepressants is secondary to enhanced β receptor stimulation by elevated synaptic levels of norepinephrine which, in turn, requires intact adrenergic nerve terminals.

In addition to β-adrenergic receptors, central α_2-adrenergic receptors have been implicated in the mechanism of action of

antidepressants. Postsynaptic α_2 receptors mediate feedback inhibition of the adrenergic cell bodies of the locus coeruleus (Svensson and Usdin, 1978), while presynaptic α_2 receptors on adrenergic nerve terminals decrease the amount of norepinephrine released into the synapse with each nerve impulse (Crews and Smith, 1978). Chronic, but not acute, administration of antidepressants results in a down-regulation of α_2 receptors both on the cell bodies of the locus coeruleus (Svensson and Usdin, 1978) and on adrenergic nerve terminals (Crews and Smith, 1978). This results in an increased rate of firing of the locus coeruleus and an increase in the amount of norepinephrine released per nerve impulse. A clinical correlation in humans is implied by the finding that α_2 receptors on platelets of depressed patients are down-regulated after chronic antidepressant therapy, and this correlates with the onset of clinical action (Garcia-Sevilla et al., 1981). Since the antidepressant-induced down-regulation of β receptors appears to be secondary to elevated synaptic levels of norepinephrine, we hypothesized that α_2 receptor down-regulation may be rate-limiting in the down-regulation of β receptors by antidepressants. To test our hypothesis, we administered several different α_2 receptor antagonists with several chemically different antidepressants (Chapter One). We found that combination therapy with α_2 receptor antagonists accelerated and enhanced the antidepressant-induced down-regulation of β receptors in brain, whereas combination therapy with α_1 receptor antagonists had no effect on β receptor down-regulation. These findings suggest that a decrease in α_2 receptor function may be the rate-limiting step in the down-regulation of β

receptors by antidepressants. In addition, our findings may have important therapeutic implications. Insofar as β receptor down-regulation is thought to mediate the clinical response to antidepressants, combination therapy of antidepressants with α_2 receptor antagonists may provide a more rapid onset therapy for depression. Furthermore, the greater degree of β receptor down-regulation seen with combination therapy may result in enhanced therapeutic action in previously nonresponsive patients.

The serotonergic nervous system in brain has also been implicated in the etiology and therapy of endogenous depression. However, the role of the serotonergic system in depression is more ambiguous than is that of the adrenergic system. Chronic administration of several chemically different antidepressant drugs has been shown to down-regulate central serotonergic $5HT_2$ receptors (Peroutka and Snyder, 1980a,b). However, chronic administration of ECT increases central $5HT_2$ receptor density (Bergstrom and Kellar, 1979). Thus, down-regulation of central $5HT_2$ receptors may be important in the mechanism of action of antidepressant drugs, but it is not a universal finding with all modes of antidepressant therapy.

The mechanism by which pharmacologic antidepressants down-regulate $5HT_2$ receptors is not known. We have studied the role of serotonergic transmission in the antidepressant-induced $5HT_2$ receptor down-regulation (Chapter Four). Our findings indicate that central $5HT_2$ receptor density is not regulated by changes in the availability of serotonin. Depletion of central stores of serotonin with reserpine did not alter cerebral cortical $5HT_2$ receptor binding. In addition,

coadministration of the $5HT_2$ receptor antagonist nefazadone did not prevent the down-regulation of central $5HT_2$ receptors by amitriptyline. Others have shown that lesions of central serotonergic terminals with p-chloroamphetamine (pCA) does not prevent the down-regulation of $5HT_2$ receptors in cerebral cortex by amitriptyline (Clements-Jewery and Robson, 1982). Taken together, these findings indicate that the down-regulation of central $5HT_2$ receptors by antidepressants is not mediated by changes in synaptic levels of serotonin.

Our findings indicate that changes in $5HT_2$ receptor density induced by antidepressants, both pharmacologic agents and ECT, may involve interactions between serotonergic and adrenergic nervous systems in brain. One such interaction may be mediated by α_2-adrenergic receptors. We have shown that coadministration of α_2 receptor antagonists with several chemically different antidepressants accelerates and enhances cerebral cortical $5HT_2$ receptor down-regulation (Chapter Two). The mechanism by which α_2 receptor antagonists accelerate the antidepressant-induced down-regulation of central $5HT_2$ receptors is not known. This effect does not appear to be secondary to the accelerated and enhanced β receptor down-regulation with combination therapy, as we have shown that $5HT_2$ receptor down-regulation is independent of β receptor down-regulation (Chapter Four). It is possible that the accelerated $5HT_2$ receptor down-regulation observed with combination therapy is secondary to direct interactions between central α_2 and $5HT_2$ receptors.

We have also found evidence for an interaction between cerebral cortical β adrenergic and $5HT_2$ receptors (Chapter Four). Stimulation of β receptors in cerebral cortical slices decreased β receptor density and increased $5HT_2$ receptor density. Studies by others have found that administration of β receptor agonists to rats enhances behavioral responses to serotonergic stimulation (Ortmann et al., 1982; Cohen et al., 1982). These behavioral responses appear to be mediated in part by $5HT_2$ receptor stimulation (Peroutka et al., 1981). It is possible that the serotonergic hyperactivity after β receptor agonist therapy is secondary to increased $5HT_2$ receptor density. In addition, the increase in central $5HT_2$ receptor density after chronic ECT treatment appears to be mediated through the adrenergic nervous system since it is prevented by depletion of central norepinephrine stores (Green et al., 1983). This ECT-induced increase in central $5HT_2$ receptor density may be mediated through a β-$5HT_2$ receptor interaction. Similar to the interaction we have demonstrated in cerebral cortical slices.

Thus, the down-regulation of $5HT_2$ receptors by antidepressant drugs may occur secondary to interactions with α_2-adrenergic receptors, while the up-regulation of $5HT_2$ receptors induced by ECT may be mediated by interactions with β-adrenergic receptors. It is a long standing clinical observation that depressed patients respond differently to the various forms of antidepressant therapy, suggesting more than one underlying mechanism of the disease. The different effects of ECT and antidepressant drugs on central $5HT_2$ receptors may, therefore, reflect the heterogeneity of endogenous depression. That

is, one subgroup of depressed patients may benefit from $5HT_2$ receptor up-regulation induced by ECT, while another subgroup may benefit from $5HT_2$ receptor down-regulation induced by pharmacologic antidepressants.

In addition to changes in $5HT_2$ receptor density, the significance of the serotonergic system in antidepressant action may be related to its interactions with the adrenergic nervous system in brain. It has been found that lesions of central serotonergic terminals with 5,7-dihydroxytryptamine prevent the down-regulation of central β receptors by several, but not all, antidepressants (Brunello et al., 1982). Thus, serotonin may play a permissive role in the down-regulation of β receptors by certain antidepressants, and the etiology and therapy of endogenous depression may be more directly related to alterations in adrenergic synaptic transmission.

We have found that in addition to β and α_2 adrenergic receptors, α_1 adrenergic receptors may be important in the etiology and therapy of depression. It has been shown that α_1 and β receptors mediate opposite electrophysiologic responses. In several brain regions, α_1 receptor stimulation is excitatory for neuronal firing, whereas β receptor stimulation is inhibitory (Menkes and Aghajanian, 1981). Thus, the net effect of adrenergic synaptic transmission appears to depend on the balance between α_1 and β adrenergic receptor responsiveness.

We have found that chronic, but not acute, administration of the prototype tricyclic antidepressant desipramine increases cerebral cortical α_1 receptor stimulated phosphatidylinositol (PI) turnover,

while it decreases cerebral cortical β receptor stimulated cAMP production (Chapter Six). The former was associated with no change in α_1 receptor density, whereas the latter was associated with a decrease in β receptor density. In contrast, we found that reserpine, an agent which precipitates depression in certain patients, increases central β receptor stimulated cAMP production, but does not alter central α_1 receptor stimulated PI turnover. Thus DMI and reserpine induce opposite changes in the relative responsiveness of α_1 and β adrenergic receptors.

While the current major hypothesis of depression focuses on β receptor sensitivity, the pathology of endogenous depression may be directly related to the balance between inhibitory β adrenergic receptors and excitatory α_1 adrenergic receptors in brain (Fig. 28). Depression may result from increased β receptor-adenylate cyclase interaction (inhibitory) relative to α_1 receptor-PI interaction (excitatory), and antidepressants may act by restoring the α_1-β receptor balance to normal. Thus, agents which shift this α_1-β receptor balance in favor of the β receptor, e.g. reserpine, would be expected to precipitate depression. In contrast, agents which shift this balance in favor of the α_1 receptor, e.g. DMI, would be expected to treat depression.

Serotonergic transmission may be indirectly related to the pathology of depression by modulating the adrenergic nervous system and thus the α_1-β receptor balance. It has been shown that depletion of central stores of serotonin increases the density of β receptor binding sites in brain. Thus, diminished serotonergic transmission

may shift the α_1-β receptor balance in favor of the β receptor and precipitate a depressive episode. Studies cited previously suggest that the serotonergic nervous system may also be important in the effects of antidepressants on the α_1-β receptor balance, in that intact serotonergic terminals are required for several antidepressants to down-regulate β receptor density, and, thus, shift the α_1-β receptor balance in favor of the excitatory α_1 receptor. Thus, while changes in both serotonergic and adrenergic transmission have been implicated in depression, the net result of these changes may be a change in the balance between central α_1 and β adrenergic receptor responsiveness. It is this balance which may be directly involved in the pathogenesis and treatment of endogenous depression.

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BIOGRAPHICAL SKETCH

James Scott was born on August 19, 1958 in Chicago, Illinois, and moved to Florida with his parents at an early age. He attended elementary school and high school in Daytona Beach, Florida, and in 1976 moved to Gainesville to attend the University of Florida, where he received a bachelors degree in chemistry in 1980. In the fall of 1980, he entered medical school at the University of Florida. During the summer after his first year of medical school, he entered the M.D.-Ph.D. program to study pharmacology and therapeutics with Dr. Fulton Crews. He received the M.D. degree in June, 1986, and subsequently began an internship in internal medicine at the University of Florida. In July, 1987, he had completed his internship training and entered residency training in neurology at the University of Florida.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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